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STUDIES IN THE GENETICS OF DROSOPHILA
VII. FURTHER ARTICLES ON GENETICS, CYTOLOGY AND TAXONOMY

Directed by

J. T. PATTERSON

Professor of Zoology
The University of Texas

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The benefits of education and of useful knowledge, generally diffused through a community, are essential to the preservation of a free government.

Sam Houston

Cultivated mind is the guardian genius of Democracy, and while guided and controlled by virtue, the noblest attribute of man It is the only dictator that freemen acknowledge, and the only security which freemen desire.

Mirabeau B. Lamar

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PREFACE

The thirteen articles in this bulletin constitute No. VII of the series of publications to be issued from our genetics laboratory since 1940. The present series of articles represents a continuation of the one published in 1949 (No. 4920), and, in the main, deals with the same topics of genetics, cytology and taxonomy of the genus Drosophila with special reference to their bearing on evolution in this genus.

J. T. PATTERSON

Austin, Texas,
December 15, 1951.

I. THE GENETIC RELATIONSHIPS OF *DROSOPHILA LITTORALIS* MEIGEN TO THE OTHER MEMBERS OF THE VIRILIS GROUP

J. T. PATTERSON

INTRODUCTION

Drosophila littoralis was described by Meigen in 1830 from Germany. In 1935 Duda reported its occurrence at Schlesien in Silesia. Since then it has been reported from a number of different places in Europe. The most extensive records are by Dr. Hans Burla (1951), who collected it over much of Switzerland and found that it was not especially rare, except in the high Alps. He states that it prefers the banks of streams, lakes, springs, and similar habitats. Although it is sometimes found near houses, yet it was never taken within dwellings. He therefore regards this species as wild-type. Other records of *littoralis* are from France (pinned specimen from Burla), Styria in Austria (Mainx, D. I. S., No. 22, 1948), and Italy, where Professor A. Buzzati-Traverso reports (personal communication) that he has collected it in several localities in the northern part of the country.

A preliminary report on the results obtained in crosses between *littoralis* and the other members of the virilis group has been included in a book by Patterson and Stone (1952). It is the purpose of the present article to give the original data, and to show that the results derived from genetic tests support the conclusions based on the cytological analysis.

The first intimation we had that *littoralis* might belong to the virilis species group came in a letter addressed to Dr. M. R. Wheeler (May 10, 1950) from Dr. Burla. Upon our request, he kindly sent us a stock of a strain from Merligen, Switzerland (No. 2000.1), and later sent a second stock of a strain from Vitznau, Switzerland (No. 2000.3). We are also indebted to Professor Buzzati-Traverso for sending us two cultures of a strain collected at Kulm Aargau in Switzerlind (No. 2096.1). These four cultures, representing three different geographical strains, constitute the material on which the following description and experimental results have been based.

POSSIBLE RELATIONSHIP OF *D. LITTORALIS* TO *D. IMERETENSIS*

In April, 1950, Professor Th. Dobzhansky informed the writer that N. N. Sokolov (1948) had described a new member of the virilis species group from Russia. Inasmuch as the publication in which his description appeared was not available here, Dr. Dobzhansky kindly translated the article and sent us a copy. In order to have a basis for comparing *D. imeretensis* with Meigen's *D. littoralis*, with the view to determine any possible relationship that might exist between the two forms, Dr. Wheeler and the writer prepared a full modern description of the latter form. Our description and Dobzhansky's translation of Sokolov's article are given below.

Drosophila littoralis Meigen 1830. (Redescribed.)

External characters of imagines.

♂. Arista with 7 branches, two below terminal fork. Antennae yellowish-gray, slightly darker apically. Face yellowish-gray, darkest on upper side of carina, which is rather broad and slightly sulcate. Front grayish-tan. Orbits and ocellar triangle more pollinose. Middle orbital about ½ length of anterior, ⅓ length of posterior. Two oral bristles, with second ⅔ first. Palpi grayish-yellow with one prominent bristle. Cheeks grayish-yellow, their greatest width about ⅓ to ¼ greatest diameter of eyes. Eyes dark red, with light colored pile.

Acrostichal hairs in 6 rows; no prescutellars. Anterior scutellars divergent. Sterno-index about .83. Mesonotum light brown, with dark brown longitudinal stripes just within the dorsocentral rows, and a less distinct stripe lateral to rows, broadly interrupted at transverse suture. Entire surface slightly pollinose. Pleurae darker than mesonotum, color about like that of stripes, suture paler; scutellum light brown. Legs pale grayish yellow. Apical bristles on first and second tibiae, preapicals on all three.

Abdomen grayish brown, pollinose, lighter on basal segment in midline.

Wings slightly dusky, veins darker; posterior crossveins with narrow dark cloud; anteriors not cloudy. Two stout bristles at distal costal break. Third costal section with heavy bristles on basal ⅔. Costal index about 2.8; 4th vein index about 1.2; 5x index about 1.2; 4c index about .66.

Length body 3.8 mm. (in live specimen); wings 3.2 mm.

♀, length body 4.0 mm.; wings 3.5 mm.

Internal characters of imagines.

Testes in male of four days light orange, with three inner coils and basal ½ of first outer coil light orange, other three and ½ outer coils of gyres transparent yellowish. In old males the entire testes becomes orange.

Spermathecae lightly scleritized, with small wart-like spines on apical half. Ventral receptacle with about 50 loose coils.

Other characteristics, relationship and distribution.

Eggs.—4 filaments.

Puparia.—The freshly formed puparium is white, soon turning red, and finally becoming tannish red. Horns are short, about 1/25 length of puparium. Anterior spiracle with about 12 branches. The anal pore, which is characteristic of the puparia of other members of the group, is also present in this species. The larvae pupate in or at the margin of the food.

Chromosomes.—The metaphase plate shows two pairs of rods, a pair of large V's, a pair of J's and a pair of dots. The salivary gland nuclei have six long strands and a dot (C. L. Ward). The rod-shaped X has a subterminal centromere, and the Y is J-shaped. It has been determined cytologically that the large autosomal V is the result of fusion between chromosomes 3 and 4, while the autosomal J is due to a pericentric inversion in chromosome 2.

Distribution.—As was pointed out above, the known distribution of this species lies in Europe within the western part of the Palaearctic Region.

Drosophila imeretensis Sokolov 1948. (Translation by Dr. Th. Dobzhansky.)

In 1940 I collected a single female, and in 1946 several dozen specimens of Drosophilas in Kutais and its neighborhood, which resembled *Drosophila virilis*. A further study has shown that we were dealing with a distinct species which we named *Drosophila imeretensis species nova,* according to the region where this form was first discovered (Imeretia, Georgian SSR).

Individuals of *Drosophila imeretensis* are large in size, 3–3.5 mm. Their color is dark. Antennae light brown, aristae with 5 upper and 2 lower branches. Head dark brown, carina nose-like, weakly furrowed in the middle. Cheeks yellowish grey, rather narrow, ¼ to ⅓ of the transverse eye diameter. Second bristle of the vibrissa no longer than half of the first. Second orbital situated almost at the level of the first, laterally from the latter and ⅓ as long as the latter. The parts of the head near the orbital bristles much lighter (nearly pigmentless) than the rest of the area.

Eyes large with a short pile, dark red, acquiring a yellowish tinge with age of the type of "prune." In old specimens collected in nature the eye color sometimes approaches the type of "sepia."

Thorax yellowish brown with 4 rather distinct dark stripes; two of the latter in the middle broader, beginning at the anterior margin and continuing along the entire length and passing onto the scutellum, where they fuse into a broad stripe. The two narrower stripes are placed laterally from the median stripes, and beginning at the suture, reach the posterior margin of the throax and go over to the lateral margins of the scutellum. As a result of this, the anterior scutellar bristles are located on dark spots. In front of the suture the thorax has diffuse dark spots.

The distance between the anterior and posterior dorsocentrals is less than between the right and left dorsocentrals. Six rows of acrostichals are present between the anterior dorsocentrals.

Legs yellow, the first tarsal joint longer than the three following.

Wings grey, strongly clouded at the anterior and posterior crossveins. The longitudinal veins mostly dark, the costal vein being the lightest and the fourth one the darkest. The dark pigmentation is intensified at the distal ends of the II, III and IV veins. On the contrary, the distal end of the V vein is devoid of dark pigment, the boundary between the pigmented and the unpigmented parts being sufficiently sharp. In old flies, especially those caught in nature, the dark pigmentation on the distal margin of the wing passes over onto the membrane, giving rise to a clear clouding near the veins.

Some specimens caught in nature have the dark pigmentation even more strongly developed on the wing membrane, forming a definite pattern along the veins, resembling that in the mutation "ebony" in *Drosophila melanogaster*.

The wing indices are as follows: costal, 2.9; IV vein, 1.6; index IV, costal 0.8; index V, large crossvein 1.2.

Abdomen dark, with light stripes on the margin of the tergites.

Female genitalia resembles those of *Drosophila virilis*, but the ovipositor is nearly hidden and has on the vaginal plates 3 rather large, thick teeth and also 15 smaller teeth on the external margin of the plate. Most of these teeth (about 10) are on the very margin of the plate, and the remainder a bit off the margin. The length ratios of the three longest teeth are about 5:4:3.5. Thus the shortest of them is more than half as long as the longest.

Spermatheca is rather large, has the form of a truncated ellipsoid, and on a considerable portion of the surface is streaked with oblique streaks.

Testes orange-red, visible through the abdominal wall. External genitalia smallish, clasper with 8 teeth on average, varying from 6 to 10.

The egg of *Drosophila imeretensis* has four filaments, the length of the shortest pair being about ¾ of that of the egg.

In the laboratory the larvae of this species tend to pupate either in the food or immediately above its surface. Pupa color red.

The chromosomal complement contains 5 pairs of chromosomes; 2 pairs are rod-shaped, one being clearly shorter than the other, and apart from them are one pair of V-shaped chromosomes, one pair with submedian centromeres, and one pair of microchromosomes. The male has a Y-chromosome with submedian centromere.

A series of characters indicates that our new species is close to the so-called wild forms of the virilis group (Patterson).

Drosophila imeretensis crosses with great difficulty to *D. virilis*. As a result of many trials we succeeded in getting 6 hybrid individuals, which proved to be wholly sterile.

In 1945 the species *Drosophila imeretensis* was discovered also in the northern parts of USSR (Moscow region).

A comparison of these two descriptions shows that the two forms are very similar morphologically, and are identical for several of the characters. This is true for the number of arista branches, eye color, rows of acrostichal hairs, mesonotal pattern, color of legs, color of abdomen, dusky wings, testes color, egg filaments, body length, color of puparia and method of larval pupation. The configurations of the metaphase plates are also alike, each consisting of two pairs of rods, a pair of large V's, a pair of J's and a pair of dots. The Y chromosome is J-shaped in both forms. Finally, the behavior of each form in crosses to *D. virilis* is similar. Sokolov states that *imeretensis* crosses with great difficulty to *virilis*, and only after many trials did he succeed in obtaining six completely sterile individuals. Our experience with *littoralis* was similar, except that we failed entirely to obtain any hybrids in crosses of this species to our standard Pasadena stock of *virilis*, perhaps because we did not use enough flies. Some differences between the two descriptions are indicated, but such differences are no greater than would be expected when two parties, working independently, describe the same species.

Our conclusion is that *littoralis* and *imeretensis* are strikingly similar and may represent geographical strains of the same species, or else they are closely related sibling species. This point cannot be determined without access to living material of the Russian form.

CROSSES BETWEEN *D. LITTORALIS* AND OTHER MEMBERS OF THE GROUP

Reciprocal crosses between *littoralis* and eight other forms of the virilis group were carried out, making a total of sixteen separate matings (Table 1). In making up the cultures for these tests, ten pairs were placed in each large vial (35 × 100 mm.), and the flies transferred to fresh food at least once. The strains with stock numbers and places of origin are as follows:

virilis (V) ; #1801.8 from Texmelucan, Mexico.
texana (T) ; #1128.10 from New Orleans, Louisiana.
novamexicana (N) ; #1714.4 from San Antonio, New Mexico.
americana (A) ; Eastern division, #1882.6 from Millersburg, Pennsylvania; western division, #1960.81, from Poplar, Montana.
montana (M) ; #1218.8d from Cottonwood Canyon, Utah.
borealis (B) ; #2077.4b from Itasca Park, Minnesota.
flavomontana (F) ; #1950.1c from Chester, Idaho.
lacicola (Lc) ; #1360.2 from Fairbanks, Minnesota.
littoralis (Li) ; #2000.1 from Merligen, Switzerland.

In the preliminary tests of *littoralis* to *virilis* the so-called Pasadena strain of the latter was used. As noted above such matings proved to be incompatible. Thereafter the Texmelucan strain from Mexico was employed, because experience in the laboratory had shown that crosses of other members of the group often went better to this than to the Pasadena strain.

In the reciprocal matings of *littoralis* and *virilis* the cross went much better when *virilis* was used as the female parent (Table 1. 1, 2). When it was used as the male parent, 700 pairs yielded about 40 hybrids for an average of about .06 per pair, while the reciprocal cross of 920 pairs gave 1,962 hybrids, for an average of about 2.13 per pair. This is at the rate of nearly eight times higher than for the first cross. These results are in line with those obtained with many previous tests in this laboratory between *virilis* and the wild-type forms.

The reciprocal crosses between *littoralis* and *montana* gave the opposite result (3, 4). In these matings the higher production of hybrids was obtained when *littoralis* was used as the female parent. In this mating 410 pairs produced 180 hybrids for an average of .44. In the reciprocal cross, 450 pairs yielded but a single abnormal male.

The reciprocal crosses between *littoralis* and *lacicola,* which is a closely related species to *montana,* behaved similarly to those of the preceding matings. With *littoralis* as the female parent, 200 pairs gave 26 hybrids for

an average of about .12, while the same number of pairs in the reciprocal cross gave only two abnormal offsprings (5, 6).

TABLE 1

P_1 crosses between *D. littoralis* and other members of the virilis group

	Crosses ♀ ♂	Number of pairs	Number of hybrids	Females	Males
1.	Li × V	700	40	22	16
2.	V × Li	920	1962	970	992
3.	Li × M	410	180	103	77
4.	M × Li	450	1	0	1
5.	Li × Lc	220	26	15	11
6.	Lc × Li	220	2	1	1
7.	Li × F	170	0	....	....
8.	F × Li	230	279	138	141
9.	Li × B	180	0	....	....
10.	B × Li	160	4	1	3
11.	Li × A	226	2	1	1
12.	A × Li	178	0	....	....
13.	Li × T	172	0	....	....
14.	T × Li	140	0	....	....
15.	Li × N	204	0	....	....
16.	N × Li	204	0	....	....

The next four matings include the reciprocal crosses between *littoralis* and two new species of the montana complex described in the next article under the names *D. flavomontana* and *D. borealis*. The cross Li ♀ × F ♂ was incompatible (7), while 230 pairs of the reciprocal cross gave 279 offspring for an average of 1.2 per pair (8). The cross Li ♀ × B ♂ was incompatible (9) but the reciprocal cross yielded four abnormal offspring (10). The six remaining crosses listed in the table, represent the reciprocal matings between *littoralis* and *americana, texana* and *novamexicana;* all were incompatible, except for the cross Li ♀ × A ♂ which gave two abnormal hybrids.

There are listed in Table 2 twenty-seven different crosses, involving three types of F_1 hybrids; the *littoralis/virilis* hybrids (LiV), the *virilis/littoralis* hybrids (VLi) and the *littoralis/montana* hybrids (LiM). In the second column, the number of hybrids tested are given for each mating. Small mass matings were used, each containing the same number of males or females as tested hybrids. Hence, the numbers tested in this column refer to the hybrids only. In the first cross (1) 134 pairs of VLi hybrids were inbred, but the cross proved to be incompatible.

In the backcross series (2–13), each of the three types of hybrids was mated reciprocally to its parental forms. The LiV hybrid females were fertile to the males of both species, while the reciprocal matings were incompatible (2–5). The numbers tested were small, due to the difficulty of obtaining hybrids. In cross 3, eight hybrid females produced 68 offspring for an average of 8.5 per tested fly, and in cross 5, nine hybrid females gave only eight offspring for an average of .89. The VLi hybrids gave comparable results, in that the hybrid females were fertile, while matings with the hybrid males were incompatible (6–9). In cross 7, 129

tested females produced 111 offspring for an average of .86 per tested fly, and in cross 9, 124 hybrid females gave 227 offspring for an average of 1.83. Finally, the crosses with LiM hybrids followed a similar pattern, the hybrid females producing offspring, while the matings with the hybrid males were incompatible (10–13). In cross 11, 36 tested females gave 22 offspring for an average of .61 per tested fiy, and in cross 13, 60 tested females yielded only 8 offspring for an average of .13.

In this series the number of hybrids tested in backcrosses varied as did the number of offspring per tested fly. Nevertheless, the results are consistent, for, without exception, the hybrid females were fertile, at least to some degree, while the crosses with the hybrid males were all incompatible.

In the lower half of Table 2 are shown the results obtained from outcrosses of the VLi hybrids to seven different members of the virilis

TABLE 2

Tests with F_1 hybrids

	Crosses ♀ ♂	Number tested	Number of offspring	Females	Males	Type of cross
1.	VLi × VLi	134	0	...	...	inbred
2.	Li × LiV	9	0	...	...	backcross
3.	LiV × Li	8	68	35	30	backcross
4.	V × LiV	9	0	...	...	backcross
5.	LiV × V	9	8	4	4	backcross
6.	Li × VLi	180	0	...	...	backcross
7.	VLi × Li	129	111	56	55	backcross
8.	V × VLi	184	0	...	...	backcross
9.	VLi × V	124	227	121	106	backcross
10.	Li × LiM	36	0	...	...	backcross
11.	LiM × Li	36	22	12	10	backcross
12.	M × LiM	24	0	...	...	backcross
13.	LiM × M	60	8	5	3	backcross
14.	M × VLi	134	0	...	...	outcross
15.	VLi × M	124	288	153	135	outcross
16.	Lc × VLi	84	0	...	...	outcross
17.	VLi × Lc	180	10	7	3	outcross
18.	F × VLi	122	0	....	...	outcross
19.	VLi × F	123	0	...	....	outcross
20.	B × VLi	96	0	...	....	outcross
21.	VLi × B	100	0	....	....	outcross
22.	A × VLi	184	0	....	....	outcross
23.	VLi × A	216	0	....	....	outcross
24.	T × VLi	144	0	...	...	outcross
25.	VLi × T	204	99	48	51	outcross
26.	N × VLi	144	0	...	...	outcross
27.	VLi × N	120	2	2	0	outcross

group (14–27). It will be observed that, here again, all seven matings involving the use of the VLi hybrid males proved to be incompatible. But four of the seven reciprocal crosses showed some fertility. In the VLi ♀ × M ♂ cross, 124 pairs produced 288 offspring for an average 2.3 per tested female (15). With *lacicola* the VLi ♀ × Lc ♂ mating was slightly fertile. The 180 tested pairs yielded but ten offspring for the very low average of .05 (17). The crosses of the VLi females to *flavomotana* and *borealis* males (19, 21) and to *americana* (23) were all

incompatible. In cross 25 (VLi ♀ × T ♂), 204 tested pairs gave 99 offspring for an average of about .48. These hybrids were useful for determining the chromosomal relationship between *littoralis* and *texana*. Since the initial cross between these two species proved to be incompatible (Table 1. 13, 14), the (VLi) T hybrids made it possible for Hsu (Article III) to work out this relationship. In the VLi ♀ × N ♂ cross (27) 120 tested flies gave two females, both of which proved to be sterile, although they were observed mating with *novamexicana* males.

In Table 3 are listed the results obtained in backcross tests with five different types of F_2 hybrids. The number tested in each of these several crosses is relatively small, owing to the difficulty of obtaining these hybrids. The five types of hybrides are, in order, (LiV)Li (1–4), (VLi)V (5–8), (VLi)Li (9–12), (VLi)M (13–16), and (VLi)T (17–20). In crosses with the (LiV)Li hybrids only one of the four matings was fertile. This was the (LiV)Li ♀ × Li ♂ in which twelve tested hybrids gave 43 offspring for an average of 3.6 per fly. In the tests with the (VLi)V hybrids, three out of four of the matings were fertile. In two of these (6, 8) the hybrid represented the female, and in the third it represented the male (7). The average numbers of offspring for the three fertile crosses were 1.7, 7.3, and 9.8, respectively. The most interesting result is the one obtained in the cross with the hybrid male (7), for it represents the only case among twenty-three crosses listed in Tables 2 and 3 in which the hybrid male in backcross and outcross tests produced progeny.

TABLE 3

Tests with F_2 hybrids

	Crosses ♀ ♂	Number tested	Number of offspring	Females	Males	Type of cross
1.	Li × (LiV)Li	13	0	....	....	backcross
2.	(LiV)Li × Li	12	43	19	24	backcross
3.	V × (LiV)Li	12	0	....	....	backcross
4.	(LiV)Li × V	14	0	....	....	backcross
5.	Li × (VLi)V	64	0	....	....	backcross
6.	(VLi)V × Li	55	92	46	46	backcross
7.	V × (VLi)V	20	146	73	73	backcross
8.	(VLi)V × V	23	227	131	96	backcross
9.	Li × (VLi)Li	20	0	....	....	backcross
10.	(VLi)Li × Li	20	1	pupa	0	backcross
11.	V × (VLi)Li	18	0	....	....	backcross
12.	(VLi)Li × V	20	7	4	3	backcross
13.	Li × (VLi)M	36	0	....	....	backcross
14.	(VLi)M × Li	24	0	....	....	backcross
15.	M × (VLi)M	36	0	....	....	backcross
16.	(VLi)M × M	24	0	....	....	backcross
17.	Li × (VLi)T	7	0	....	....	backcross
18.	(VLi)T × Li	8	1	1	0	backcross
19.	V × (VLi)T	6	0	....	....	backcross
20.	(VLi)T × V	8	28	16	12	backcross

In the four crosses with the (VLi)Li hybrids (9–12), two showed some fertility. Thus, the (VLi)Li ♀ × Li ♂ mating yielded a single pupa from which the imago failed to emerge (10). In the other fertile cross,

(VLi) Li ♀ × V ♂, 20 tested hybrids gave seven offspring (12). In the next series of tests (13–16), which involved the reciprocal crosses of the (VLi) M hybrids to both *littoralis* and *montana*, no progeny was obtained. Finally, the (VLi) T hybrid showed some fertility when it was used as the female parent (17–20). In the test to *littoralis* males only a single female offspring was produced (18). In the other fertile cross, (VLi) T ♀ × V ♂, eight tested hybrids gave 28 offspring for an average of 3.5 per tested fly. The results recorded in Table 3 indicate that, with the exception of a few crosses, the fertility of the F_2 generation flies is very low, with little opportunity for gene exchange between the different forms. This point will be discussed in a later section.

INCOMPATIBILITY OF THE CROSSES WITH LITTORALIS

Sixty-three different crosses are listed in Tables 1 to 3, of which twenty-five resulted in the production of offspring—sometimes but a single individual. There were therefore thirty-eight crosses which failed to produce offspring, *i.e.*, the matings were "incompatible." The question is, then, To what is this incompatibility due? The tests fall into two classes: the initial or P_1 crosses, and the hybrid crosses.

The P_1 crosses

In Table 4 are listed the results obtained in dissections of females from sterile or nearly sterile P cultures (Table 1). In this series of tests the flies were tested in small mass matings beginning on the sixth day after emergence, and the females dissected eight or nine days later. The results from these several crosses will be considered in the order in which they appear in Table 4.

TABLE 4

Dissected females from sterile or nearly sterile cultures

	Crosses ♀ ♂	Females dissected	Females inseminated	Per Cent inseminated	Sperm in receptacles motile	Sperm in receptacles non-motile
1.	M × Li	48	0			
2.	Lc × Li	50	1	2	1	0
3.	Li × F	50	1	2	1	0
4.	Li × B	50	0			
5.	B × Li	50	0			
6.	Li × A	58	17	29	10	7
7.	A × Li	50	0			
8.	Li × T	50	18	36	8	10
9.	T × Li	50	0			
10.	Li × N	50	16	32	6	10
11.	N × Li	51	0			
12.	VLi × Lc	57	4	7	1	3
13.	VLi × F	50	0			
14.	VLi × B	67	51	76	14	37
15.	VLi × A	50	27	54	12	15
16.	VLi × N	50	16	32	9	7

In the first cross, M ♀ × Li ♂, none of the 48 females had been inseminated, while the corresponding P_1 cross gave one abnormal, sterile

male from 450 tested pairs. In the Lc ♀ × Li ♂ cross one of the 50 dissected females showed a few weakly motile sperm in the ventral receptacle, while the 220 tested pairs of the P_1 cross yielded two abnormal flies. One of the females of the Li ♀ × F ♂ cross contained a few non-motile sperm in the ventral receptacle. In the corresponding P_1 cross no offspring was produced by the 170 tested pairs. None of the 50 females of the Li ♀ × B ♂ cross had been inseminated, and the P_1 cross was incompatible. In the B ♀ × Li ♂ mating none of the females had been inseminated, but in the P_1 cross 160 tested pairs gave four abnormal flies.

The next six matings in the table include the reciprocal crosses between *littoralis* and the three members of the americana complex, *americana, texana* and *novamexicana*. The P_1 reciprocal matings were all incompatible, except for the Li ♀ × A ♂ cross in which 226 tested pairs yielded two abnormal flies (Table 1, 11–16). In the dissected series none of the females had been inseminated when *littoralis* was used as the male parent, but when used as the female parent, the percents inseminated were 29, 26 and 32, respectively, for *americana, texana* and *novamexicana*. In these three cases the number of females containing non-motile sperm in the receptacles is slightly higher than those having motile sperm (27 vs. 24). In most of the females with live sperm, the motility was very weak, but in the *texana* cross there was one female with very active sperm, indicating that a recent insemination had occurred. Since the males and females of these crosses had been exposed to each other for some eight or nine days, it is obvious that fertilization would occur from time to time throughout the period of exposure. Consequently, one would expect to find live sperm in the receptacles of some of the females at the time of making the dissections.

The conclusion to be drawn from these results is that the main barrier to cross-fertility between *littoralis* and other members of the virilis group is sexual isolation. But in addition to this, there is also operating in some of the crosses another mechanism which causes the inactivation or death of the sperm in the reproductive tract of the alien female. Such an isolating mechanism has been reported by us in other species groups of the genus Drosophila.

The hybrid crosses

The various types of hybrid crosses are given in Tables 2 and 3. There is a total of forty-six such crosses listed, exclusive of the inbred test. In twenty-three of these the hybrid entered the cross as the male parent, and in all except one, failed to produce progeny. This would suggest that the hybrid males were sterile in twenty-two crosses. Dissected samples of the eight different types of hybrid males used in these crosses revealed the fact that in seven of the eight types spermatogenesis was incomplete, and never progressed beyond the "sperm-bundle" stage; that is to say, they do not form functional gametes (motile sperm). In the exceptional case motile sperm were found to be present in the testes of the male hybrids. These were the (VLi)V males, which failed to produce offspring when mated to

the *littoralis* females, but were cross-fertile to the *virilis females* (Table 3. 7). It will be observed from the formula of this type of hybrid male that there was only one opportunity for recombinations of the *virilis* and *littoralis* chromosomes to occur, so that the chromosomes received by the F_3 generation flies could be predominantly of the *virilis* type.

The degenerative phenomena in spermatogenesis is not unique for these hybrid males. For example, a similar phenomenon has been observed in the hybrid males between weak strains of *pseudoobscura* and *persimilis* (Dobzhansky, 1934), and in those between the subspecies of *pallidipennis* and *centralis* (Patterson and Dobzhansky, 1945), to mention a couple of examples.

It was not determined whether the sterile hybrid males of *littoralis* mate with normal females, as was found to be the case with such males in certain crosses between members of the mulleri subgroup where the presence of the insemination reaction made such determinations a simple matter (Patterson 1946, 1947). In that subgroup the sperm-free semen caused a reaction similar to that produced by the normal semen. The insemination reaction is not very pronounced and is more or less transitory among members of the virilis group (Wheeler 1947). Hence, any attempt to determine whether the sterile hybrid males of *littoralis* ever mate with normal females would require a great amount of direct observation.

Dissections were made of the VLi females that had been outcrossed to five different types of males but had produced no or only a few progeny. The males used were those of *lacicola, flavomontana, borealis, americana* and *novamexicana.* The results obtained in these dissections are listed at the bottom of Table 4 (12–16). The VLi ♀ × Lc ♂ cross had yielded seven females and three males from 180 tested pairs. Fifty-seven VLi females were dissected, and four were found to have been inseminated, with the receptacles of one containing motile and three non-motile sperm (Table 4, 12). The VLi ♀ × F ♂ cross was incompatible, and none of the 50 dissected females had been fertilized (Table 4, 13). The third cross, VLi ♀ × B ♂, was also incompatible, but 51 of the 67 dissected females had been fertilized, with 14 containing motile and 37 non-motile sperm in their receptacles (Table 4, 14). The cultures of the original matings did not produce any offspring (Table 2, 21), nor did those of this dissected series. Evidently, the sperm are completely inactivated in the reproductive tract of the alien females. In the VLi ♀ × A ♂ cross, 27 of the 50 dissected females were found to have been inseminated, with 12 containing motile and 15 non-motile sperm (Table 4, 15). In one of the cultures a few larvae developed and eventually a single sterile male emerged from a pupa, although in the original test no progeny developed (Table 2, 23). Finally, 16 of the 50 dissected females of the VLI ♀ × N ♂ cross were found to have been fertilized, with 9 containing motile and 7 non-motile sperm in their receptacles (Table 4, 16). These cultures yielded two females and six males, while the original mating produced two females (Table 2, 27). These flies proved to be sterile.

DISCUSSION AND CONCLUSIONS

Article III of this publication contains a detailed account of the relationships, based on cytology, of *littoralis* to the other members of the virilis group. A general account of the evolution of this group will be found in Chapter X of "Evolution in the Genus Drosophila" (Patterson and Stone, 1952). The general outline of the cytological evolution is quite clear. It shows that the cytological ancestor must have been *Drosophila virilis*, or else a virilis-like form differing by a single inversion in chromosome 2. The latter form gave rise to all of the known wild species of the group. One line acquired two overlapping X chromosome inversions and split into *texana, novamexicana* and *americana.* A second line acquired two additional inversions in chromosome 2, one of which was the characteristic pericentric inversion, as well as three inversions in chromosome 4. On the one hand, this latter stem acquired a number of inversions and gave rise to *littoralis* in Europe, and, on the other hand, it acquired many additional inversions and gave rise to the montana complex in America. *Drosophila virilis* is close to the central position of the whole group.

In addition to the inversion differences, there are characteristic lines which are revealed in the metaphase patterns. Thus, *virilis* and *novamexicana* have the typical primitive configuration of five rods and a dot, and the subspecies *texana* and *americana* share the 2–3 fusion, with the latter form having the X–4 fusion. The montana complex has no fusions, while *littoralis* has an independent 3–4 fusion.

With reference to the relationship of *littoralis* to the other members of the group, the results from the genetic tests closely parallel the findings of cytology. This is indicated by the data on the P_1 crosses (Table 1). It is cross-fertile to *virilis*, which, on the basis of the cytology, represents the postulated ancestral form. The reciprocal matings both produce offspring, although the cross goes better when *littoralis* represents the male parent. With the exception of a small amount or residual fertility with *americana* (Li ♀ × A ♂), crosses to members of the americana complex are incompatible. This complex evolved in and is confined to the Nearctic region, where it has developed almost complete sexual isolation from its European relative. The genetic tests show a much closer relationship of *littoralis* to members of the montana complex than to those of the americana complex. The montana complex is a second line of descent which evolved in the Nearctic region, and the four members crossed to *littoralis* all showed some degree of cross-fertility, usually when *littoralis* is used as the female parent.

REFERENCES

Burla, Hans. 1951. Systematik, Verbreitung und Oekologie der Drosophila-Arten der Schweiz. Rev. Suis. d. Zool., 58, No. 2, 23–175.

Dobzhansky, Th. 1934. Studies in hybrid sterility. I. Spermatogenesis in pure and hybrid *Drosophila pseudoobscura*. Z. Z.m. A., 21:443–446.

Duda, O. 1935. Drosophilidae. In Linder, E. Die Fliegen der Palaearktischen Region, 58g:1–118.

Hsu, T. C. 1952. Chromosomal variation and evolution in the virilis group of Drosophila. This publication, Article III.

Meigen, J. W. 1830. Systematische Beschreibung der bekannen europäischen zweiflügelingen Insekten. 6:81.

Patterson, J. T. 1946. A new type of isolating mechanism in Drosophila. Proc. Nat. Acad. Sci., 32:202–208.

Patterson, J. T. 1947. The insemination reaction and its bearing on the problem of speciation in the mulleri subgroup. Univ. Tex. Publ., 4720:41–77.

Patterson, J. T., and Th. Dobzhansky. 1945. Incipient reproductive isolation between two subspecies of *Drosophila pallidipennis*. Genetics, 30:429–438.

Patterson, J. T., and W. S. Stone. 1952. Evolution in the Genus Drosophila. Macmillan Company, New York (in press).

Sokolov, N. N. 1948. A new species of Drosophila—*Drosophila imeretensis*. Comptes Rendus (Dokl.) Acad. Sci. U.S.S.R., 59, No. 5: 1007–1008.

Wheeler, M. R. 1947. The insemination reaction in intraspecific matings of Drosophila. Univ. Tex. Publ., 4720:78–115.

II. REVISION OF THE MONTANA COMPLEX OF THE VIRILIS SPECIES GROUP

J. T. PATTERSON

INTRODUCTION

We have shown that the virilis group of species falls into four main divisions (Patterson and Stone, 1952). These are 1, *Drosophila virilis* Sturtevant which occurs in the Eastern Palaearctic and Oriental Regions of the Eastern Hemisphere and the Nearctic and Neotropical Regions of the Western Hemisphere; 2, *D.americana americana* Spencer, and *D.americana texana* Patterson, Stone and Griffen, and *D.novamexicana* Patterson, all three of which are confined to the Nearctic Region; 3, *D.montana* Patterson and Wheeler and *D.lacicola* Patterson, which are also confined to the Nearctic Region; and 4, *D.littoralis* Meigen and *D.imeretensis* Sokolov which are European forms. In a recent report on a detailed study of chromosomal inversions of members of this group, Dr. T. C. Hsu (Article III) has designated two forms of the montana complex as yampa and superior strains. These are locality names and refer to the Yampa River in Colorado and to Superior, Wisconsin. The taxonomic ranks of these two strains have not been definitely worked out, and it is one of the purposes of the present article to determine their ranks. They are described below under the names *Drosophila flavomontana* and *Drosophila borealis*, respectively.

DESCRIPTION OF NEW SPECIES

Drosophila borealis, sp. nov.

External characters of imagines.

♂, ♀. Arista with about 8 branches including the terminal fork. Front dull brownish black, ocellar triangle and orbits thickly pollinose. Antennae dark brown, 3rd joint a little paler, 2nd joint with two stronger bristles. Face dark brown, tannish in antennal foveae; carina prominent, broad and flat, faintly sulcate. Anterior reclinate orbital small, about ⅓ length proclinate, ⅕ length posterior reclinate. One pair of strong orals, quite long and cruciate; 2nd oral thin, little less than ½ length 1st. Cheeks, below the row of orals, as dark as face, below eyes distinctly lighter tan; cheek width nearly ¼ greatest diameter of eye. Eyes dark red with dense black pile.

Acrostichals in 6 rows, the outer rows often irregular and appearing as 8 rows; no prescutellars. Anterior scutellars divergent. Mesonotum and scutellum dull dark brown to blackish brown with distinct lighter brown pollinose stripes in dorsocentral rows and along median two rows of acrostichals. Pleurae darker, especially below, less pollinose than mesonotum. Sterno-index about 0.8. Legs brownish, darkest on fore coxae and femora and all tibiae, other regions lighter. Apicals on 1st and 2nd tibiae, preapicals on all three.

Abdominal tergites solid color, very dark blackish brown, thinly pollinose and slightly shining; sternites dark.

Wings nearly clear, veins brown, posterior crossveins clouded. Costal index about 2.2; 4th vein index about 1.6; 5x index about 1.1; 4c index about .7. Two well-developed bristles at apex of first costal section. Third costal section with heavy bristles on its basal ⅔.

♂. Length body 3.0 mm. (in live specimens); wing 3.0 mm.

♀. Length body 3.0 mm.; wing 3.0 mm.

Internal characters of imagines.

Testes orange with 4 inner and 6 outer coils or gyres.

Spermathecae sclerotized, strawberry shaped, with spinules on its upper half; ventral receptacle long spiral with about 40 coils.

Other characteristics, relationship, and distribution.

Eggs.—4 filaments with dorsal pair terminating in hooks.

Puparia.—Basic color light brownish, turning darker in older specimens. Each anterior spiracle with 11 branches; horn-index about 11.

Chromosomes.—Metaphase plate has 4 pairs of rods, one pair of small V's and a pair of dots. The V-shaped element is chromosome 2, the result of a pericentric inversion. Salivary gland nuclei show six long strands and a dot.

Relationship.—Belongs to the montana complex of the virilis group.

Distribution.—Has been identified cytogically by Hsu from Chester, Idaho, and Hamilton, Colorado, where it coexists with *D.flavomontana;* and at Itasca Park in Minnesota and at Lake Croix, Wisconsin, where it coexists with *D.lacicola.*

Types: Holotype male, four paratype males and 5 paratype females (No. 2077.4b) from a stock originating from Itasca Park, Minnesota. Types deposited in The University of Texas collection.

Notes: Dr. H. T. Spieth in the summer of 1950 reared this species from larvae and pupae taken from the decaying phloem tissue of the Aspen, *Populus tremuloides,* at Itasca Park, Minnesota.

Drosophila flavomontana, sp. nov.

External characters of imagines.

♂, ♀. Arista with about 7 branches including the terminal fork, rarely with 8 branches. Front brownish tan, ocellar area darkened, the triangle and orbits thickly pollinose. Antennae light tan, 3rd joint a little darker, 2nd joint with two stout bristles. Face tan, carina darker gray, especially above. Carina high, wide, with a well-developed median sulcus. Palpi yellowish tan, each with a fine apical bristle. Ocellar bristles long. Anterior reclinate orbital ⅓ length proclinate, ¼ length posterior reclinate. One pair of strong orals, 2nd oral quite thin, about ¼ length 1st. Cheeks pale tan, distinctly yellowish below eyes; eyes small, the cheek width nearly ⅓ greatest eye diameter. Eyes dark red with short, thick, light-colored pile.

Acrostichals clearly in 6 rows; no prescutellars. Anterior scutellars divergent. Mesonotum pale tan with brownish cast, heavily overlaid with

tan pollen along median four acrostichal rows, along dorsocentral rows and above notopleural suture; the general effect is a light tan thorax with darker brown stripes just within each dorsocentral row with parallel stripes laterally, broken at the transverse suture. Scutellum largely tan, the basal median area lighter pollinose. Pleurae noticeably darker than mesonotum; a wide, indefinite dark burnt brown stripe from propleura across mesopleura and most of the pteropleura, with a lighter area just below and separating this region from the still darker sternopleura; humeral callus pale. Legs pale yellowish tan. Apicals on 1st and 2nd tibiae, preapicals on all three.

Abdominal tergites uniformly solid brown with pale pollinosity, only slightly shining. The apical, non-haired margins are pale and usually visible between segments, especially on females. Sternites darkened but lighter than dorsum.

Wings slightly dusky, veins yellowish, posterior crossveins clouded. Costal index about 3.0; 4th vein index 1.6; 5x index about 0.9; 4c index about 0.5. Two well-developed bristles at apex of first costal section. Third costal section with heavy bristles on its basal 3/5.

♂. Length body 3.2 mm. (in live specimens); wing 3.0 mm.

♀. Length body 3.3 mm.; wing 3.3 mm.

Internal characters of imagines.

Testes bright orange, with 3 inner and 5 outer coils or gyres.

Spermathecae spherical, sclerotized. Ventral receptacle long spiral with about 50 coils.

Other characteristics, relationship, and distribution.

Eggs.—4 curved filaments without hooks at ends.

Puparia.—Light brown, turning darker in older specimens. Each anterior spiracle with 11 branches; horn-index about 16.

Chromosomes.—Metaphase plate has four pairs of rods, one pair of small V's, representing chromosome 2 with pericentric inversion, and a pair of dots. The salivary gland nuclei have six long strands and a dot.

Relationship.—Belongs to the montana complex of the virilis species group.

Distribution.—This species has been collected in five different states, Idaho, Wyoming, Utah, Colorado and New Mexico, at thirteen different localities. It sometimes coexists with its nearest relative, *Drosophila montana,* with which it occasionally hybridizes in nature.

Types.—Holotype male, 4 paratype males and 5 paratype females (No. 1950.1c) from stock originally collected at Chester, Idaho, deposited in The University of Texas collection.

FIELD RECORDS OF MEMBERS OF THE MONTANA COMPLEX

In Table 1 are listed all of our field records for the different members of the montana complex. For each collection it gives the lot number, name of the person in charge of the trip, locality, number of specimens taken,

names of species indicated by symbols, and date on which the collection was made. The first collecting trip, in which *Drosophila montana* was found and recognized as an undescribed form, was made in July and August 1941 by G. B. Mainland and M. R. Wheeler. They collected a total of 270 specimens of the complex at eight different localities in three states, Colorado, Wyoming and Utah. They recognized two different color phases, "dark" and "light" (yellowish), and made up a series of dark and light pairs and sent them to the laboratory. In the first full description of *D. montana* the following comment appears: "This species has two color phases, light and dark. The description is based on the darker form. The light form has a color pattern similar to that of *D.novamericana*, but it has the same metaphase configuration of chromosomes as the darker form." (Patterson and Wheeler, 1942). Thus, it was recognized from the first that the populations of *D.montana* were not homogeneous.

TABLE 1

Collection records of members of the montana complex: *montana* (M); *flavomontana* (F); *borealis* (B); *lacicola* (Lc)

Lot No.	Collector	Locality	Specimens	Species	Date
1204.8	Mainland	Estes Park, Colorado	2	F	7-17-41
1210.8	Mainland	Grand Teton Park, Wyoming	172	M & F	7-20-41
1211.5	Mainland	Iron Creek, Yellowstone	72	M & F	7-24-41
1212.5	Mainland	Madison River, Yellowstone	12	M & F	7-25-41
1215.7	Mainland	Ogden River, Utah	1	F	7-28-41
1218.8	Mainland	Cottonwood Canyon, Utah	8	M & F	7-29-41
1220.2	Mainland	Puffer Lake, Utah	2	M	7-31-41
1223.10	Mainland	Zion National Park, Utah	1	F	8- 3-41
1228.1	Dobzhansky	Park Range, Utah	1	F	9-17-41
1228.2	Dobzhansky	Pikes Peak, Colorado	1	M	9-17-41
.....	Dobzhansky	Mather, California	stock	M	9-17-41
1285.16	Mainland	White Water Camp, New Mexico	1	M	10-20-41
1318.8	Mainland	Hart Prairie, Arizona	6	M	7- 1-42
1324.8	Mainland	Bonita Canyon, New Mexico	28	M	7- 7-42
1360.1	Anderson	Fairbanks, Minnesota	2	Lc	7-15-42
1360.1	Anderson	Hibbing, Minnesota	1	Lc	8-11-42
1360.2	Anderson	Duluth, Minnesota	2	Lc	8-11-42
1360.3	Anderson	Pequot, Minnesota	1	Lc	9-12-42
1360.4	Anderson	St. Peter, Minnesota	1	Lc	9-12-42
1363.1	Anderson	Duluth, Minnesota	2	Lc	8-17-42
1755.4	Wheeler	Lake St. Croix, Wisconsin	9	Lc & B	7-22-47
1756.2	Wheeler	Fenske Lake, Minnesota	3	Lc	7-22-47
1757.5	Wheeler	Lake Bemidji, Minnesota	4	Lc	7-24-47
1761.9	Wheeler	Chinook, Montana	1	C	7-27-47
1762.8	Wheeler	Glacier National Park, Mon.	3	C	7-28-47
1764.3	Wheeler	Bonners Ferry, Idaho	13	M	7-30-47
1767.5	Wheeler	New Meadows, Idaho	7	M	8- 1-47
1769.1	Wheeler	Grand Teton Park, Wyoming	9	M	8- 3-47
1770.3	Wheeler	Grand Teton Park, Wyoming	7	M	8- 5-47
1772.4	Wheeler	Pactola, South Dakota	2	C	8- 7-47
1862.2	Wheeler	Lake Tahoe, California	20	M	8-17-48
1942.6	Hsu	Verdi, Nevada	57	M	7-31-49
1943.6	Hsu	Truckee River, California	10	M	8- 2-49
1947.3	Hsu	Emmett, Idaho	3	F	8- 8-49
1948.4	Hsu	Carey, Idaho	2	F	8- 8-49
1949.4	Hsu	Hot Springs, Yellowstone	1	F	8-10-49
1950.1	Hsu	Chester, Idaho	13	B & F	8-11-49
1951.1	Hsu	Hamilton, Colorado	28	B & F	8-13-49
1952.2	Hsu	Antlers, Colorado	2	M	8-14-49
1953.8	Hsu	Carbondale, Colorado	9	F	8-15-49
1956.5	Hsu	Wolf Creek, Colorado	13	M	8-18-49

TABLE 1—(Continued)

Collection records of members of the montana complex: *montana* (M); *flavomontana* (F); *borealis* (B); *lacicola* (Lc)

Lot No.	Collector	Locality	Specimens	Species	Date
1957.3	Hsu	Chama, New Mexico	20	F	8-19-49
1960.1	Hsu	Cowles, New Mexico	7	C	8-21-49
2059.1	Wheeler	Duchesne, Utah	1	C	8-12-50
2064.2	Wheeler	Lander, Wyoming	7	M	9-16-50
2072.4	Wheeler	Raton, New Mexico	6	M	8-26-50
2077.4	Spieth	Itasca Park, Minnesota	39+	B	8-12-50
2077.4	Spieth	Itasca Park, Minnesota	1	Lc	8-12-50
2169.10	Wheeler	Payson, Arizona	1	M	6-24-51
2179.5	Wheeler	Crescent City, California	68	M	7-24-51
2181.2	Wheeler	Willow Creek, California	37	M	7-26-51
2182.6	Wheeler	Goldbeach, Oregon	11	M	7-27-51
2183.6	Wheeler	Elkton, Oregon	11	M	7-28-51
2184.3	Wheeler	Lake Creek, Oregon	44	M	7-29-51
2185.5	Wheeler	Carlton, Oregon	194	M	7-31-51
2186.9	Wheeler	Raymond, Washington	1	M	8- 1-51
2188.9	Wheeler	Bogachiel Park, Washington	16	M	8- 2-51
2189.4	Wheeler	Sequim, Washington	12	M	8- 3-51
2190.6	Wheeler	Verlot, Washington	15	M	8- 4-51
2191.13	Wheeler	Wenatchee, Washington	5	M	8- 5-51
2192.4	Wheeler	Carson, Washington	5	M	8- 6-51
2193.4	Wheeler	Mt. Hood Nat. Forest, Oregon	32	M	8- 7-51
2194.5	Wheeler	Bend, Oregon	13	M	8- 8-51
2195.5	Wheeler	Jackson, Wyoming	3	M	8-10-51
2196.2	Wheeler	Moose, Wyoming	24	M	8-11-51
2199.1	Wheeler	Morgan, Utah	203	F	8-13-51
2199.7	Wheeler	Morgan, Utah	1	M	8-13-51
2200.6	Wheeler	Junction, Utah	1	F	8-14-51

Upon receiving the pairs of flies, together with some individuals not mated, the writer carried out various tests, and, among other facts, recorded the color of the offspring and that of their puparia. Since it is rather difficult to establish a stock of *montana* from a single female, or even from a pair of flies, not all of the matings yielded progeny. Nevertheless, over sixty stocks were finally established in the laboratory. The data obtained in these tests have never been published. They are summarized in Table 2.

TABLE 2

Analysis of the collections of montana strains taken in July and August, 1941, by G. B. Mainland and M. R. Wheeler.

Locality and Lot Number		Number ♀♀	Number ♂♂	Dark	Light	Remarks
Estes Park, Colorado	(1204)	0	2		2	failed to breed
Teton Park, Wyoming	(1210)	97	75	74	28	hybrids present
Iron Creek, Wyoming	(1211)	35	37	30	+	hybrids present
Madison River, Wyo.	(1212)	7	5	6	+	hybrids present
Ogden River, Utah	(1215)	1	0		1	fertile to light ♂
Cott. Canyon, Utah	(1218)	5	3	4	4	all fertile
Puffer Lake, Utah	(1220)	1	1	....	2	♀ fertile, ♂ lost
Zion Nat. Park, Utah	(1223)	0	1		1	fertile to light ♀
Totals		147	124	114	38+	

It is interesting to note that the first specimens collected did not belong to the true *montana*. These are the two light males taken at Estes Park,

Colorado, which failed to produce progeny when mated to dark females from Teton Park. It is now recognized that these two specimens represented *D.flavomontana*. Of the 172 flies from Teton Park (1210), 74 dark and 28 light specimens were tested and the results demonstrated that hybrids were present in that locality. In the Iron Creek lot (1211), thirty dark flies were tested and produced results which indicated the presence of hybrids, thus showing that *flavomontana* coexisted with *montana*. The same is true for the lot from Madison River (1212). The single light female from Ogden River (1215) was virgin, and when mated to a light male from lot 1218 was fully fertile. She was therefore a *flavomontana*. Of the eight flies from Cottonwood Canyon, four were dark and four were light. These were all fertile, the dark flies producing black pupae, and the light ones red pupae. This indicates that both forms were present at this locality. The male and female from Puffer Lake were very light colored flies. The male escaped at the time of capture, but the female was fertile and produced offspring with red puparia. This would usually indicate that the fly was *flavomontana*, but it is interpreted as a *montana* because of its gene sequence. The single light male from Zion National Park (1223) belonged to *flavomontana*, since it was fertile to light females from lot 1210. Further comments on some of these cases are given in the section on genetic tests.

A few other records were added in 1941. Thus, Professor Th. Dobzhansky sent two preserved specimens, one from Park Range in Utah, which proved to be the light form, the other a specimen of *montana* from Pikes Peak, Colorado. He also sent a stock of *montana* derived from flies collected at Mather, California. In 1941 and 1942 Dr. G. B. Mainland added records of *montana* from New Mexico and Arizona. But the most important material for the year 1942 was the addition of a new form *(D.lacicola)*, which had been collected by Mr. R. C. Anderson in the lake regions of Minnesota and sent in by Dr. M. M. Green of the University of Minnesota. In 1947 Dr. M. R. Wheeler made further collections in Minnesota and Wisconsin of *D.lacicola*, and obtained other members of the complex from Montana, Idaho, Wyoming and South Dakota. Most of these specimens represented *D.montana*, but a few of them could not be identified with certainty from the records and are therefore listed under "C" (montana complex). In 1948 Wheeler added records of 20 specimens of *D.montana* from Lake Tahoe, California.

The next most important collections were made in the summer of 1949 by Mr. T. C. Hsu, accompanied by Professor M. J. D. White. This trip covered parts of Nevada, California, Idaho, Wyoming, Colorado and New Mexico, and yielded a total of 165 specimens belonging to the montana complex. The specimens identified were *montana*, *flavomontana* and *borealis*, with seven specimens from Cowles, New Mexico, not definitely identified. It will be observed that *borealis* and *flavomontana* were found to coexist at Chester, Idaho and Hamilton, Colorado.

In 1950 Dr. Wheeler obtained a single unidentified specimen of the montana complex at Duchesne, Utah, seven specimens of *montana* at

Lander, Wyoming, and six at Raton, New Mexico. Dr. H. T. Spieth of the College of the City of New York made a very extensive collection at Itasca Park, Minnesota. According to Hsu's cytological analysis, there were 39 females of *D.borealis* (number of males not recorded) and a single specimen of *D.lacicola.*

In the summer of 1951 Dr. Wheeler and Mr. W. B. Heed made two collecting trips: the first to New Mexico and Arizona, where a single specimen of *montana* was taken at Payson, Arizona; the second to the northwestern part of the United States, where collections were made in California, Oregon, Washington, Wyoming, and Utah. The trip yielded the unusually large number of 696 specimens of the montana complex. The most interesting part of this collection came from the northwestern tip of California, and the western halves of Oregon and Washington, where a total of 464 specimens of *montana* were taken at fourteen different localities, two in California and six each in Oregon and Washington. These flies probably represent a different geographical strain of *montana,* for on the average, they are larger than specimens from the high Rocky Mountains, and were found in the Coastal and Cascade Ranges at much lower elevations, sometimes at not over 100 feet above sea level. Stocks of these strains are now being studied in the laboratory.

GEOGRAPHICAL DISTRIBUTION OF THE DIFFERENT SPECIES

The localities at which the different species have been collected are plotted on the map shown in Figure 1. *Drosophila montana* has been collected at thirty-seven localities (including two in Yellowstone Park) in ten different states: Idaho, Wyoming, California, Nevada, Oregon, Washington, Utah, Colorado, Arizona and New Mexico. The main distribution range of this species follows the Rocky Mountains from Northern Idaho to as far south as Arizona and New Mexico. It occurs in these mountains at relatively high altitudes, from five to ten thousand feet, and usually above six thousand feet. In addition, it has been collected at high elevations at four localities in the Sierra Nevada Range in Western Nevada and Eastern California. As pointed out above, this species occurs at much lower altitudes in the Coastal and Cascade Ranges. As indicated in Table 1, *montana* has been found to coexist with *flavomontana* at five different localities, as follows: Grand Teton Park in Wyoming, Iron Creek and Madison River in Yellowstone Park, and Cottonwood Canyon and Morgan in Utah. It is known that in some of these places natural hybridization has taken place between these two species.

Drosophila flavomontana has been collected in five states: Idaho, Wyoming, Utah, Colorado and New Mexico, at seventeen different localities. Its distribution range overlaps that of *montana,* and, as pointed out above, the two species coexist at five different places. It also coexists with *borealis* in the Rocky Mountain Range at two localities: Chester, Idaho, and Hamilton, Colorado. However, its population density is very much less than that of *montana,* except at Morgan, Utah, where 203 specimens of *flavomontana* and a single one of *montana* were taken in the traps.

Drosophila borealis has been recorded from at least four different localities. In addition to Chester, Idaho, and Hamilton, Colorado, this species was taken in considerable numbers at Itasca Park, Minnesota by Dr. H. T. Spieth, where it coexists with *D.lacicola*. It has also been collected at Lake St. Croix in Wisconsin, where it probably coexisted with *lacicola*. The collection from Lake St. Croix was made by Wheeler and Cowan on July 22, 1947, and consisted of five females and four males. When the collection was received in the laboratory some of the flies were identified by the writer as *lacicola*. Stocks were established at that time, but unfortunately, only one (1755.4e) was still alive when Hsu checked the stock in 1951 and found that it represented *borealis*. On the basis of present knowledge the distribution range of this species appears to be discontinuous and widely scattered. There is a possibility, however, that it may have been collected between Minnesota and Idaho.

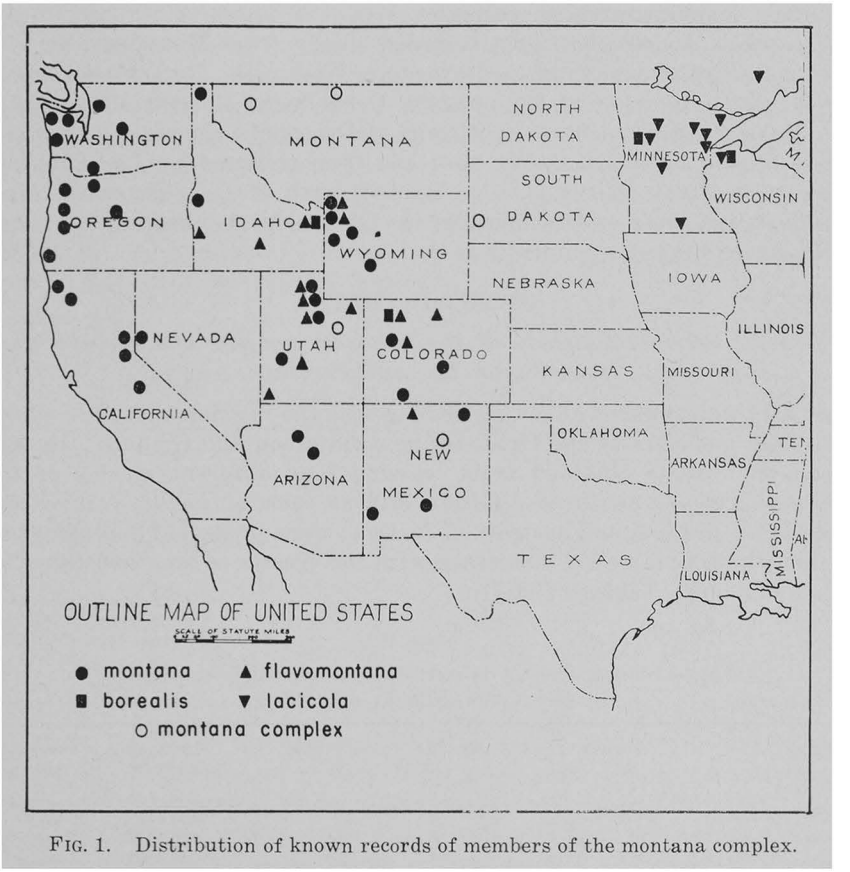


FIG. 1. Distribution of known records of members of the montana complex.

The distribution map shows five locations (circles) at which some or all of the specimens were not definitely identified. Of these five localities, three occur between Minnesota and Idaho, one in South Dakota and two in northern Montana. The collection from Pactola, South Dakota (No.

1772.4) consisted of two females which were recorded in the field notes as "lacicola-like." Each of these was mated to males of *lacicola*, but both failed to produce offspring. Because of the close resemblance of *lacicola* and *borealis*, these two specimens could very well have been the latter species. The collection from Chinook, Montana (No. 1761.9) contained a mixture of *D.americana* and three small lacicola-like flies, which failed to breed. These too could have been *borealis*. The collection from Glacier National Park in northwestern Montana (No. 1762.8) contained a pair of dark flies which failed to breed, and they likewise could have been *borealis*. Of the two remaining unidentified collections, the one from Duchesne, Utah (No. 2059.1), consisted of a single wild-type female which failed to breed when mated to the males of both *montana* and *novamexicana*. Finally, the collection from Cowles, New Mexico (No. 1960.1) contained seven flies which are recorded in the notes with the statement, "identity unknown, mantana complex species."

Drosophila lacicola has been recorded chiefly from Minnesota, with at least a few specimens from northwestern Wisconsin. Recently Dr. S. G. Smith of the Division of Entomology, Department of Agriculture, Sault Ste. Marie, Ontario, sent us specimens of Drosophila among which was a single female of *lacicola*. The flies had been collected by Dr. Th. Dobzhansky at Black Sturgeon Lake, located north of Lake Superior. This locality is indicated on the map, Fig. 1. Obviously, the distribution range of this species extends up into Canada.

GENETIC TESTS

1. *Crosses between members of the montana complex and certain other species of the virilis group*

In 1944 we published an article dealing with the relationships of *montana* and other members of the virilis group (Patterson and Griffen). In that paper, the results obtained from crossing nine different strains of the montana complex to three different strains each of *virilis*, *texana* and *americana*, and to a single strain of *lacicola*, were presented and analyzed. In this connection we are concerned with the crosses of *montana* to *virilis* and *texana* only (Tables 3 and 4).

TABLE 3

Crosses between strains of the montana complex and those of *D.virilis* (modified after Patterson and Griffen)

Strains ——> ↓	Pasadena ♀	Pasadena ♂	Henly ♀	Henly ♂	Mukden ♀	Mukden ♂	Origin of montanas	Color of pupae
1210.83; *montana*	36%	st.	2%	st.	6%	st.	Teton Park	dark
1210.98; *flavomontana*	29%	st.	4%	st.	3%	st.	Teton Park	red
1211.51; hybrid	48%	st.	1%	st.	2%	st.	Teton Park	d.& red
1211.58; hybrid	13%	st.	13%	st.	12%	st.	Iron Creek	dark
1212.5c; *montana*	27%	st.	14%	st.	2%	st.	Mad. River	dark
1218.8d; *montana*	54%	st.	18%	1%	24%	st.	Cott. Canyon	dark
1220.2; *montana*	54%	1%	29%	st.	22%	st.	Puffer Lake	red
1318.8a; *montana*	34%	1%	21%	st.	28%	1%	Hart Prairie	dark
1324.8m; *montana*	23%	st.	18%	1%	5%	st.	Bonita Can.	dark

In Table 3 are listed the data on the crosses between the montana strains and those of *virilis*. The three virilis strains used were Pasadena (originally from New York), Henly from Texas and Mukden from Manchuria. The first column of the table gives the stock numbers of the nine *montana* strains; the second, third and fourth the results from the crosses between these strains and the three virilis strains; the fifth shows the place of origin of the stocks; and the sixth indicates the color of the pupae of the offspring from the original pair matings. Stock 1210.83 was a *montana*, while 1210.98 is now known to have represented *flavomontana*. The next two stocks were hybrid strains, the first of which showed segregation of the pupal color in the offspring of the pair matings. The remaining five all belonged to *montana*. For the three virilis strains most of the crosses went reasonably well when *montana* was used as the male parent, but very poorly, or not at all, when it was used as the female parent. In the latter type of mating, 22 out of 27 crosses proved to be incompatible.

In the texana series the strains used originated from flies collected at Lake McKethan, Florida, Georgetown, Texas, and New Orleans, Louisiana. It is clear that the percents fertile in this series are very much lower than those obtained in the virilis series. In fact, exactly one-half of the matings proved to be incompatible, with 8 out of 27 when montana entered the cross as the male parent, and 19 out of 27 when it represented the female parent. Even matings producing offspring gave very low percents fertile (Table 4). It is especially interesting that all six crosses involving the stock of *flavomontana* were incompatible.

TABLE 4

Crosses between strains of the montana complex and those of *D.texana* (modified after Patterson and Griffen)

Strains ——> ↓	Lake McKethan		Georgetown		New Orleans	
	♀	♂	♀	♂	♀	♂
1210.83; *montana*	1%	st.	5%	st.	4%	st.
1210.98; *flavomontana*	st.	st.	st.	st.	st.	st.
1211.51; hybrid	st.	st.	st.	st.	4%	st.
1211.58; hybrid	2%	st.	st.	st.	1%	st.
1212.5c; *montana*	6%	st.	6%	st.	1%	st.
1218.8d; *montana*	12%	1%	1%	1%	2%	st.
1220.2; *montana*	2%	1%	1%	st.	st.	st.
1318.8a; *montana*	4%	4%	2%	1%	1%	1%
1324.8m; *montana*	2%	1%	3%	st.	st.	3%

In 1944 Dr. Mary Warters published an article in which is included an analysis of the chromosomal variability in several strains of the montana complex. It is somewhat difficult to correlate her results with those of Dr. Hsu (next article), owing to the fact that they were working on different problems; Hsu was concerned with chromosomal evolution, while Warters was interested in the amount of variability. Nevertheless, it is possible to determine the nature of most of the strains analyzed by Warters from her recorded data, combined with the writer's experimental data referred to above. Unfortunately, all except one of the original stocks have since

been lost. Warters used two different strains as standard; 1210.98 from Teton Park which, as we have indicated, was a *flavomontana,* and 1212.5c which was a true *montana.* Our interpretation of her results are summarized in Table 5. In part this table gives the original data of the writer, including the color of the flies and that of the pupae of their offspring.

Warters checked eleven dark and two light strains from Teton Park. Among the dark forms, seven represented *montana,* one was *flavomontana* (1210.87), one was hybrid (1210.131) and two could not be determined because their records are incomplete. The two light strains represented *flavomontana* (1210.98, a standard, and 1210.111). Nine dark strains from Iron Creek were checked, and of these, seven belonged to *montana* and two were hybrid (1211.55, 1211.58). One of the two dark strains from Cottonwood Canyon was checked and represents *montana.* The light strain from Puffer Lake (1220.2) is interpreted as belonging to *montana.* The two light strains from Cottonwood Canyon and the single one from Ogden River were not examined by Warters, but all three gave light offspring and red pupae, and on this basis were probably *flavomontana.*

Further comments seem desirable for some few of the strains listed in Table 5. The strains of *flavomontana* are usually light with red pupae,

TABLE 5

Matings of *montana* in field: From Teton Park, Wyoming; Iron Creek and Madison River, Yellowstone Park; Cottonwood Canyon, Puffer Lake and Ogden River, Utah; Bonita Canyon, New Mexico.

Place	Color of flies	Number tested	Number fertile	Color of pupae	Checked by Warters	Strains		
						montana	flavo-montana	hybrids
Teton Park	dark	38	31	dark	9(+2?)	7	1	1
Teton Park	light	14	14	red	2	..	2	..
Iron Creek	dark	13	12	dark	9	7	..	2
Madison Riv.	dark	6	6	dark	4	3	..	1
Cott. Canyon	dark	2	2	dark	1	1	..	..
Cott. Canyon	light	2	2	red	0	..	..	..
Puffer Lake	light	1	1	red	1	1	..	..
Ogden River	light	1	1	red	0	..	..	..
Bonita Can.	dark	1	1	dark	1	1	..	..
Totals	.....	78	70	.....	27(+2?)	20	3	4

while those of *montana* are dark with dark or black pupae. In the list there are two exceptions to this rule. The dark strain 1210.87 when tested to 1210.98 gave a gene sequence of *flavomontana,* and probably arose through hybridization. Number 1220.2 was a very light strain with red pupae, but when tested to 1210.98 showed a gene sequence like that of *montana.* It too may have arisen through hybridization, but another possible explanation is that it represents a mutation affecting the color of both the fly and the pupae. Four cases of hybrids are listed in the table. Strain 1210.131 when tested to 1210.98 showed that the X was *flavomontana,* chromosomes 3 and 5 *montana* and chromosome 4 heterozygous. The stock was established from a pair of dark flies, and therefore the hybridization must have occurred at least two generations earlier. In strain 1211.55 the tests show

that chromosomes X and 5 were *montana*, 3 and 4 *flavomontana*, with the origin of hybridization uncertain. In strain 1211.58 the X was *flavomontana*, 3 and 5 *montana* and 4 heterozygous; explanation is the same as for strain 1210.131. Strain 1212.5g had a *flavomontana* X, and 3, 4 and 5 *montana;* origin of hybridization uncertain. A few other stocks were probably hybrid, but the data are not sufficient for a satisfactory determination.

2. *Crosses of flavomontana and borealis to certain members of the virilis group*

In Table 6 are listed the results obtained in crosses of *flavomontana* and *borealis* to four other members of the virilis group. The tests were made

TABLE 6

Test crosses of *flavomontana* and *borealis*

	Crosses ♀ ♂	Number tested	Offspring ♀♀	Offspring ♂♂	Females dissected	Number & % inseminated		Sperm mot.,	Sperm non-mot.	Fertility of hybrids ♀♀	Fertility of hybrids ♂♂
1.	F × V	153	4	3	50	9	18%	1	8	st.	st.
2.	V × F	198	110	89	50	26	52%	5	21	fert.	st.
3.	F × M	150	34	42	50	12	24%	8	4	fert.	st.
4.	M × F	200	1	2	50	1	2%	0	1	st.	st.
5.	F × Lc	153	68	44	50	25	50%	13	12	fert.	st.
6.	Lc × F	164	35	24	50	22	44%	5	17	fert.	st.
7.	F × Li	185	146	140	50	38	76%	22	16	fert.	st.
8.	Li × F	170	0	0	50	1	2%	1	0	....	.
9.	F × B	150	0	0	50	2	4%	0	2	....	....
10.	B × F	200	0	0	50	1	2%	0	1	....	....
11.	B × V	270	1	2	50	4	8%	1	3	st.	st.
12.	V × B	248	9	2	60	40	67%	10	30	st.	st.
13.	B × M	186	14	7	50	18	36%	5	13	fert.	st.
14.	M × B	220	2	0	50	11	22%	3	8	non-viable	
15.	B × Lc	248	0	0	50	0	....	....	....	....	....
16.	Lc × B	222	0	0	50	0	....	....	....	....	.
17.	B × Li	160	1	3	50	0	....	....	....	abnormal	
18.	Li × B	180	0	0	50	0	....	....	....	....	....

in large vials, each containing ten females and ten males of the two species under test. The flies were aged six or seven days before the crosses were made. At least 50 females from each cross were dissected after an exposure of five or six days, and the number and percent inseminated determined. The table shows the number of pairs tested, the number of offspring produced (if any), the number of females dissected, the number and percent inseminated, the condition of the sperm in the reproductive tract of such females, and the fertility of the F_1 hybrids. The following stocks were used:

virilis (V), Stock 1801.1 from Texmelucan, Mexico
montana (M), Stock 1218.8d from Cottonwood Canyon, Utah
lacicola (Lc), Stock 1360.2 from Fairbanks, Minnesota
littoralis (Li), Stock 2000.1 from Merligen, Switzerland
flavomontana (F), Stock 1950.1c from Chester, Idaho
borealis (B), Stock 2077.4b from Itasca Park, Minnesota

In making tests for fertility, the F_1 hybrids were backcrossed to the two parental forms. The results obtained in such tests are shown in the last column of the table. In referring to the numbers tested in the second column, we shall use the phrase "pairs tested," even though the crosses were made in small mass matings.

The reciprocal crosses between *flavomontana* and *virilis* went much better when *virilis* was used as the female parent (crosses 1 and 2). The 153 tested pairs of the F ♀ × V ♂ cross yielded but seven offspring. Nine of the dissected females had been inseminated (18%), but the sperm were not motile in eight of these. Only three of the seven hybrids lived long enough to be tested for fertility, one female and two males. The single female and two male hybrids were sterile. In the V ♀ × F ♂ cross, 26 of the dissected females had been inseminated (52%), with the sperm immobilized in 21 specimens. This mating yielded 199 hybrids. Backcross tests showed that at least some of the females were fertile, but none of the males produced offspring. Their testes were found to be rudimentary.

In the crosses between *flavomontana* and *montana*, the matings went better when the former species was used as the female parent (crosses 3 and 4). Thus in the F ♀ × M ♂ cross, 150 tested pairs produced a total of 76 hybrids and 12 of the dissected females were found to have been inseminated (24%), with eight containing motile sperm in their receptacles. Backcross tests demonstrated that the hybrid females were fertile and the males sterile. In the reciprocal cross, 200 tested pairs gave three hybrids, one female and two males. All of these were found to be sterile. Only one of the 50 dissected females contained sperm in her reproductive tract, and none of these was motile.

Both reciprocal crosses between *flavomontana* and *lacicola* produced a fair number of hybrids (crosses 5 and 6). In the F ♀ × Lc ♂ cross, 153 tested pairs yielded 112 offspring, 68 females and 44 males. Twenty-five of the 50 dissected females (50%) had been inseminated, with 13 containing motile and 12 immobilized sperm in their receptacles. The female hybrids were fertile when mated to *lacicola* males, but failed to produce offspring to *flavomontana* males. In the reciprocal cross, 164 tested pairs produced 59 hybrids. Twenty-two of the 50 dissected females had been inseminated, but only five of these contained motile sperm in their receptacles. In the backcross tests the females proved to be fertile, while the males were sterile.

Only one of the reciprocal crosses between *flavomontana* and *littoralis* yielded offspring (crosses 7 and 8). This was the F ♀ × Li ♂ mating, in which 185 tested pairs yielded 286 hybrids. Thirty-eight of the 50 dissected females (76%) had been inseminated, with 22 containing motile sperm in their receptacles. In the backcross tests the hybrid females were fertile when mated to *littoralis*, but failed to produce offspring when mated to *flavomontana* males. In the reciprocal cross, 170 tested pairs failed to produce hybrids, and only one of the 50 dissected females had been inseminated.

The reciprocal matings between *flavomontana* and *borealis* were both incompatible, with only three of the 100 dissected females containing sperm in their receptacles, all immobilized (crosses 9 and 10). The crosses between *borealis* and *virilis* gave a few hybrids, with the mating going better when *virilis* was employed as the female parent (crosses 11 and 12). In this cross 40 of the 60 dissected females had been inseminated for a percentage of 67. In ten of the females the sperm were motile. All hybrids from both crosses were sterile in backcross.

In the crosses between *borealis* and *montana* the B ♀ × M ♂ cross gave 21 hybrids, and 5 of the 18 inseminated females had motile sperm in their receptacles (crosses 13 and 14). In the backcross females proved to be fertile, while the males were sterile. The M ♀ × B ♂ cross yielded but two females; one dying in the puparium and the other soon after emergence.

The last four matings represent the reciprocal crosses between *borealis* and *lacicola* and *borealis* and *littoralis*. Three of the four crosses were incompatible, and the fourth, B ♀ × Li ♂, produced four abnormal flies. None of the 200 dissected females had been inseminated.

SUMMARY AND CONCLUSIONS

The results obtained in the genetic tests, displayed in Table 6, reveal several points of interest. Six of the 18 P_1 crosses were incompatible (8,9,10,15,16,18), and two others produced non-viable or abnormal zygotes (14,17). Of the ten crosses which gave viable offspring, the backcrosses to the parental types of four were incompatible for both sexes (1,4,11,12). In the six remaining crosses, the female hybrids were fertile, while the male hybrids failed to produce offspring in backcross. This would indicate that all male hybrids are sterile. This conclusion is supported from dissections of samples of such males. Without a single exception the testes of these males contained no live or fully-formed sperm. Instead, their contents were composed of strands and debris from disintegrating elements. In this connection it should be pointed out that the number of individuals of the second generation was always relatively small as compared to the number produced in several of the P_1 crosses.

These facts raise the question as to what extent the different members of the montana complex might exchange genes in nature. Since such exchanges could only be possible between the species crosses which produce fertile hybrids, they would be limited to the six combinations which gave fertile F_1 females, as follows: V × F, F × M, F × Lc, Lc × F, F × Li and B × M.

Gene flow between *virilis* and *flavomontana* is practically impossible, for in addition to having a small, scattered population, *virilis* is ecologically isolated from all the other members of the group, due to its domestic type of habitat.

Of the two reciprocal crosses between *flavomontana* and *montana*, the F × M mating gives fertile female hybrids, thus making possible gene exchange between the two species. Although our records show that these two forms are usually found at separate places, yet they are sympatric in at least five different localities of the Rocky Mountain region (Table 1).

As we have shown above, natural hybridization does occur between the two species. However, any subsequent gene exchange must occur through crosses between the female hybrids and *montana* males.

Both reciprocal crosses between *flavomontana* and *lacicola* give fertile female hybrids. The female hybrids from the F × Lc cross were fertile in backcross to *lacicola* males, but failed to produce offspring to the *flavomontana* males. The female hybrids from the Lc × F cross were fertile to both types of parental males. Whether these two species would exchange genes cannot be answered, because their populations are not in contact. Hence in all probability they are geographically isolated (Fig. 1).

In the crosses between *flavomontana* and *littoralis* the F × Li mating gave a relatively high number of hybrids, of which the females were fertile in backcross to *littoralis* males, but not to *flavomontana* males. These two species are isolated geographically, since *littoralis* occurs in Europe while *flavomontana* is restricted to the Nearctic region. Hence, natural hybridization between them cannot occur. It should be stated that in the single fertile backcross only a very few F_2 generation flies were obtained.

The only fertile hybrids obtained between *borealis* and the other five species to which it was tested were in the B × M cross, which yielded a few fertile females. This species is largely iolated from the other forms of the complex. Our records show that it is sympatric with *flavomontana* at two localities in Colorado, and with *lacicola* at one locality each in Minnesota and Wisconsin. However, it is reproductively isolated from both of these species, due mainly to the incompatibility of the P_1 crosses. The only possible opportunity for gene exchanges to occur between *borealis* and any other member of the montana complex might be with *montana* but, according to our records, the two species have never been collected at the same locality.

An analysis of the data listed in Table 6 indicates that sexual isolation and the inactivation of the sperm in the reproductive tract of the alien female are mechanisms mainly responsible for the failure of over half of the crosses to produce hybrids. There is also a certain amount of mortality among the developing zygotes, as indicated by the presence of dead larvae in some of the cultures.

Members of the American montana complex and the European *littoralis* arose from the Primitive III stem in the virilis group ancestry (see Hsu, next article). The members of this complex have diverged extensively both cytologically and genetically, and despite a slight residual crossfertility between them, they have evolved into quite distinct species.

REFERENCES

Hsu, T. C., 1952. Chromosomal variation and evolution in the virilis group of Drosophila. Article III, This Puplication.

Patterson, J. T., and A. B. Griffen, 1944. Relationships of *Drosophila montana* and *D. lacicola* to other members of the virlis group. Univ. Tex. Publ., 4445:194–211.

Patterson, J. T., and W. S. Stone, 1952. Evolution in the genus Drosophila. Macmillan Company. In press.

Patterson, J. T., and M. R. Wheeler, 1942. Description of new species of the subgenera Hirtodrosophila and Drosophila. Univ. Tex. Publ., 4213:67–109.

Warters, Mary, 1944. Chromosomal aberrations in wild populations of Drosophila. Univ. Tex. Publ., 4445:129–174.

III. CHROMOSOMAL VARIATION AND EVOLUTION IN THE VIRILIS GROUP OF DROSOPHILA

T. C. Hsu

INTRODUCTION

The problem of evolution has been attacked from various angles. During recent years, many authors have reviewed the progress of different fields of approach (Dobzhansky, 1941, from the standpoint of genetics; Mayr, 1942, from that of systematics; Simpson, 1944, from that of paleontology; and White, 1945, from that of animal cytology).

Of particular interest is the field of cytogenetics which was developed along with the genetical and cytological achievements. Species relationships can be studied not only by comparison of morphological characters, but also by direct studies through species crosses. The characters of the hybrids will sometimes throw new light which ordinary methods could not reveal, for example, the behavior of the chromosomes.

A good deal of work on plant material has been accumulated. Due to the fact that polyploidy is common in plants, fertile hybrids are rendered possible by simple doubling of the chromosomes. The classical case of an artificial species, *Primula kewensis*, is just one among many of such examples. Amphidiploidy is also very common under natural conditions. Thus in Gossypium, Nicotiana, Triticum, Brassica, and many other plant groups, amphidiploidy is one of the most efficient ways of creating new species in Nature.

However, polyploidy is not so common and hybrids between species are not so easily obtained in the animal kingdom as in plants. Comparatively fewer cases of interspecific hybridization are known in animals than in plants; even fewer cases are fully investigated. Owing to the presence of the giant salivary chromosome, Dipterous insects are of especial value for cytological analysis. But so far, only the *melanogaster-simulans* and the *pseudoobscura* section of Drosophila and a few other cases in Sciara and Drosophila have been studied more extensively with respect to the cytological analysis (for fuller review, see Patterson, 1942a; Wharton, 1942, 1943; Warters, 1944; Patterson and Stone, 1952).

Inversions or other rearrangements are present, not only in the interspecific hybrids, but also in populations of the same species. The extensive work of Dobzhansky and his colleagues, Stalker and Carson, and Dubinin and Sokolov, has shown that some rearrangements may have adaptive values. Therefore, the relation of chromosomal changes to an understanding of evolution is of increasing importance.

Geneticists, cytologists, and evolutionists are looking for a group of species which possesses a number of characteristics that facilitate this type of evolutional study. First of all, this group should consist of a number of species, for a monotypic species can tell little about the trend of speciation. However, most large groups of species are not suitable for our

purpose because they are either too difficult to breed or are not cross fertile. In Drosophila, some species groups, such as melanogaster, have a good many species, but unfortunately most of the species are cross incompatible. Apparently the species in these groups (with but a few exceptions) are not very closely related or they have developed strong isolating mechanisms between them which prevent cross-breeding. The virilis group is excellent. It has a number of good species as well as subspecies, and each form is cross fertile to at least one of the other members in the group. Further, most of the species are not difficult to breed in the laboratory. Cytologically the species differ in chromosome rearrangements which are completely traceable in at least the americana complex; and it is still possible to analyze the montana complex, although the problem is more complicated.

A brief survey of the previous investigations and the present situation of the virilis group indicates that *Drosophila virilis* Sturtevant (1916) was not the first species in this group described by taxonomists. More than a century ago, Meigen (1830) described *Drosophila littoralis*, but it was only quite recently that Burla (1949, D.I.S., 23:87, 1951) suggested that this species belongs in the virilis group. Therefore since the publication of descriptions of *virilis*, it had been the only species of its kindred recognized until 1936 when Spencer discovered a close relative in swampy places in Ohio, U. S. A. The new form, known as *D. virilis americana*, was regarded as a subspecies of *virilis* by the describer. It differs from *virilis* in a number of morphological characters (Spencer, 1938). Cytologically, the two forms also differ in karyotypes (Hughes, 1939a) and in some gene rearrangements (Hughes, 1939b). *Drosophila virilis* has for its metaphase chromosome configuration five pairs of rods and a pair of dots, while *americana* has two pairs of V's, a pair of rods and a pair of dots. The X-chromosome of *americana* is fused with the 4th of *virilis* to form a V (Patterson, Stone and Griffen, 1940; and Stalker, 1940). The other V-pair represents the fusion of chromosomes 2 and 3 which are independent elements in *virilis*. Patterson, Stone and Griffen (1940) found another form related to *virilis* and *americana* in Texas, *D. virilis texana*, also at first regarded as a subspecies of *virilis*. It has one pair of V's, three pairs of rods and a pair of dots, the X-chromosome is not included in the V. These authors made extensive genetical studies on the three forms.

Patterson (1941) and Patterson and Wheeler (1942) described two new species of the virilis group, namely, *D. novamexicana* Patterson and *D. montana* Patterson and Wheeler, respectively. The former species has a metaphase configuration similar to that of *virilis*, i.e., five pairs of rods and a pair of dots; but the latter species has its second chromosome V-shaped. Simultaneously, Patterson (1942a) and Patterson, Stone and Griffen (1942) suggested from their extensive data on inter- and intraspecific variations, crossfertility, isolation and species relationships that these forms, including *americana* and *texana*, were all of species rank. Thus by 1942, the virilis group contained five species. Further, these authors suggested that *americana* had originated by hybridization between *texana* and *novamexicana*.

In 1944, Patterson described *D. lacicola*, which has the same metaphase configuration as montana, but differs in other characteristics. Stone and Patterson (1947), using a multiple mutant stock of *virilis*, proved that the V-shaped chromosome of *texana* is a result of fusion between the *virilis* chromosome 2 and 3; thus both *texana* and *americana* have this fusion while *americana* has the X-4 fusion in addition. Moreover, since the discovery of natural hybrids between *americana* and *texana* in the overlapping distribution zone and the low degree of isolation between the two forms, they revised the species list of the virilis group as follows:

I. *D. virilis* Sturtevant
II. *D. montana* Patterson and Wheeler
III. *D. lacicola* Patterson
IV. (a) *D. americana americana* Spencer
(b) *D. americana texana* Patterson, Stone and Griffen
V. *D. novamexicana* Patterson

Therefore *americana* and *texana* are considered as two subspecies of the species *Drosophila americana*.

In the original description of *Drosophila montana*, Patterson and Wheeler (1942) include the comment: "This species has two color phases, light and dark. The description is based on the darker form. The light form has a color pattern similar to that of *D. novamexicana*, but it has the same metaphase configuration as the darker form." In 1944, Warters analyzed strains of montana from different localities and found that it is an extremely variable species as far as chromosome arrangements are concerned. Unfortunately, with exception of one stock, all the other strains have been lost. The stock which survived has been used to clarify the old data of Warters by hybridization with the new stocks that have been collected by this laboratory since 1947. Among these, we found that there are more than a single species. The taxonomic and genetic accounts on the relatives of *montana* is presented by Prof. Patterson (Article II). Here we shall state only that there are four forms involved in the montana complex, namely, the typical *D. montana*, the yellowish *D. montana* yampa strains (the light form of the original description), *D. lacicola*, and the small, dark-bodied *D. montana* superior strains.

European authors have included two old world forms in the virilis group: *D. imeretensis* Sokolov (1948) and *D. littoralis* Meigen (Burla, l.c.). Detail genetic work on *littoralis* is presented by Dr. Patterson in Article I.

To date we have in the virilis group the following nine forms:

I. (1) *D. virilis*
II. The americana complex
(2) *D. novamexicana*
(3) *D. americana americana*
(4) *D. americana texana*
III. (5) *D. littoralis*
IV. The montana complex
(6) *D. montana*
(7) *D. montana* yampa strains
(8) *D. montana* superior strains
(9) *D. lacicola*

The purpose of this article is to disclose and to interpret the evolutionary changes in the chromosomes by means of salivary gland chromosome analysis.

MATERIAL AND METHODS

The strains used in the present study were mainly collected and maintained by the Genetics Laboratory, the University of Texas. A few strains were supplied by other institutions or persons. The number and the localities of the strains will be presented individually in the tables later.

For each species or subspecies a homozygous strain was chosen as the test strain when possible. However, since we have no homozygous strains for *D. littoralis* and *D. lacicola,* a strain with more than one inversion in heterozygous state was used in each case. The test strains for the several species or subspecies are:

Drosophila virilis	Pasadena stock
Drosophila novamexicana	1720.9a, Gila River, New Mexico
Drosophila americana americana	Anderson, Indiana
Drosophila americana texana	1128.10, New Orleans, Louisiana
Drosophila montana	1218.8d, Cottonwood Canyon, Utah
Drosophila montana yampa strains	1953.8a, Carbondale, Colorado
Drosophila montana superior strains	1951.1h, Chester, Idaho
Drosophila lacicola	1360.1, Fairbanks, Minnesota
Drosophila littoralis	2000.1, Merligen, Switzerland

The test strains were tested against *virilis,* and to one other if possible, to determine the relationships in the chromosome patterns. Individual strains of each species were tested against their respective test strains to get the variations of the chromosome rearrangements.

The task of determining whether a strain is americana or texana is rather difficult. The identity of each strain was determined by checking metaphase configuration of the brain ganglia of six larvae. If all six showed the metaphase configuration of one subspecies, the stock was described as a pure strain of that subspecies. On the other hand, if one or more larvae showed the karyotype of the other subspecies or a hybrid configuration, the strain was regarded as a hybrid. But six larvae may not be sufficient to warrant a definite conclusion in all cases, since there is still a possibility that a hybrid stock could show one type of configuration in the six slides. A few of the strains were thus rechecked by the genetic method used by Stone and Patterson (1947).

Each strain was crossed to its respective test strain. The salivary gland chromosomes of the offspring were examined on at least six female larvae in each case to detect the presence of rearrangements. Again there is a chance that a heterozygous strain may show only one kind of the rearrangements in the six slides (about 1 in 32 as calculated by Dobzhansky, 1948). However, as the present work is not as critical as those on population mathematics, the error would influence the result only slightly.

Using the salivary gland chromosome map of *virilis* as the standard, each inversion found was given a special symbol, e.g., 2a, 3b, 4j, etc. For each inversion, the breakage points were determined as closely as possible. It is extremely difficult to determine all the points of breakages, especially when several inversions occur together much of the time. For the analysis of the strains under each species or subspecies, we attempted to make a basic formula of inversions; and additional inversions found in any strain are recorded in the variation tables individually. Salivary gland smear preparations were made according to the method suggested by Hsu (1947, D.I.S., 21:90), but using orcein instead of carmine.

EXPERIMENTAL

Drosophila virilis Sturtevant

The Pasadena stock of *Drosophila virilis* was used as the standard of all the forms. This does not necessarily mean, however, that it is the most primitive species of this group. This stock is vigorous, and its females are rather acceptable to foreign males.

As a rule cytologists do not find it convenient to use the salivary gland chromosome map of other workers. So far, we have four salivary gland chromosome maps of *virilis* in the literature (Fujii, 1936; Hughes, 1939b; Patterson, Stone and Griffen, 1940; and Fujii, 1942). Among these maps, that of Griffen is the most natural and detailed one. If *virilis* chromosomes are observed under phase contrasting microscope, they look very similar to those of Griffen's map. However, perhaps due to the pretreatment with HCl, we could not follow his map band by band, and were obliged to make a map for our purpose, which is used here (Fig. 13).

Cytological analysis made by Griffen (Patterson, Stone and Griffen, 1942) and Warters (1. c.) have shown a consistent chromosome pattern of *virilis*, with no aberrations among more than 4,000 chromosomes observed (also cf. Fujii, 1936; Chino and Kikkawa, 1933). A few additional strains tested by us also agree with the findings of other workers. *Drosophila virilis* is, therefore, a remarkably stable species cytologically.

THE AMERICANA COMPLEX

The americana complex consists of three forms, namely, *D. novamexicana, D. americana americana* and *D. americana texana.* Extensive collection data made by this laboratory together with records from some other institutions have shown the wide distribution of this complex. In these records *texana* has been recorded from all the southeastern states, including Florida, Georgia, Tennessee, Arkansas, North Carolina, Virginia, Alabama, Mississippi, Louisiana, Oklahoma and central and eastern Texas. *Drosophila novamexicana* has been collected from New Mexico, Arizona and Colorado. *Drosophila americana americana* has the widest distribution area, both east and west: the eastern branch includes central Texas, Arkansas, Tennessee, North Carolina, Virginia, Missouri, Illinois,

Indiana, Ohio, Michigan, Vermont, New York and Pennsylvania; and the western branch has been collected from Kansas, South Dakota, Nebraska and Montana. All the flies from the eastern branch are dark-bodied and produce dark offspring when crossed with *texana*, and on the other hand, the western branch has in addition light colored strains which produce yellowish offspring when crossed with *novamexicana*.

The two subspecies *americana* and *texana* have a long, belt-like overlapping zone from central Texas through Arkansas and Tennessee to North Carolina and Virginia. In the overlapping zone natural hybrids of these two forms occur (Stone and Patterson, 1947).

Cytological analyses have shown that against *virilis* these three forms have a number of inversions in common. Therefore we will not describe the inversions separately under each species.

I. Types of Inversions.

A number of the inversions found in this complex have been described or figured elsewhere by other authors. Brief descriptions for all the inversions that could be found in this complex are presented below.

Xa Always associated with Xb or Xbc. Found in all the strains of the three forms.

Xb Overlaps Xa above. Found in all the strains of the three forms.

Xc Overlaps Xa and Xb above. Found in all the *novamexicana*, most of *americana* and a few *texana* strains.

Xd A small inversion included in Xc. Found in some *americana* strains from Chadron, Nebraska.

2a Submedian. Found in all the strains of the three forms.

2b Subterminal. Found in all the *novamexicana* and a few western *americana* strains.

2c A large submedian inversion including 2a within its boundary. Found in all the *novamexicana* and a few western *americana* strains.

3a Almost basal. Found in all the *novamexicana* and a few western *americana* strains.

4a Submedian. Found in all the *novamexicana*, many *americana* and some *texana* strains.

4b A short segment reinverted within the boundary of 4a. Found in many *americana* and some *texana* strains.

5a Submedian. Found in most *texana* and a number of eastern *americana* strains.

5b Subterminal. Found in *novamexicana*, a number of *americana* and a few *texana* strains.

II. *Drosophila novamexicana* Patterson.

The test strain (1720.9a, from Gila River, N.M.) gives the following inversions when crossed with *virilis:* Xabc, 2abc, 3a, 4a, and 5b.

Since the breakages of 2c are just outside the boundary of those of 2a, inversion 2a thus has been brought back to its original *virilis* sequence

in *novamexicana*. In *virilis/novamexicana* hybrids, we can often observe two unpaired short segments, one near the base and the other on the middle. Occasionally cells with good pairing condition reveal the existence of the included inversions.

The chromosomes of all the available strains tested were proven to be identical to the test strain. These strains are:

1714.4	San Antonio, New Mexico	1952.2	Antlers, Garfield Co., Col.
1720.9b	Gila River, New Mexico	1954.3a	Whitewater, Mesa Co., Col.
1853.11	Cave Creek, Arizona	1954.3b	Whitewater, Mesa Co., Col.

In other words, there has been found no variation in the chromosomes of this species.

III. *Drosophila americana americana* Spencer.

The test strain of *americana* employed is a stock from Anderson, Indiana. In hybrids with *virilis*, there are three inversions on the X (a, b and c) and one each on chromosomes 2 and 5 (2a and 5a). Its inversion formula is therefore rather simple, being Xabc, 2a, 3, 4, 5a, 6. In tests using this stock, *americana/novamexicana* hybrids give no inversions on the X, two inversions on the second (2b and 2c), one each on 3 and 4 (3a and 4a, respectively) and two on 5 (5a and 5b).

TABLE 1

Inversion Variations in *Drosophila americana americana*

Basic formula: Xabc, 2a, 3, 4, 5(a or b);
..., chromosomes identical to the basic formula;
/, heterozygous for the respective inversion;
*, data from Warters, 1944.

Strain No.	Locality	Eastern Strains X	2	3	4	5
*	Smithville, Ohio	...	...	...	-/ab	a/b
*	Peewee 1, Ohio	...	...	...	-/ab	a/b
*	Peewee 2, Ohio	...	...	...	...	b
*	Peewee 3, Ohio	...	...	...	-/ab	a
*	Overton, Ohio	...	...	...	-/ab	a/b
*	Independence 2, Ohio	...	...	...	...	a
*	Independence 3, Ohio	...	...	...	...	b
*	Independence 4, Ohio	...	...	...	-/ab	a/b
*	St. Mary's Riv., Ohio	...	...	...	...	a/b
*	South Licking 1, Ohio	...	...	...	...	a/b
*	South Licking 2, Ohio	-/ab	...	...	...	a
*1277.8	Eva, Tenn.	-/ab	...	...	...	a/b
*1278.14b	Eva, Tenn.	...	...	...	...	a
*1271.13b	Hiwassee Resv., Tenn.	...	...	...	-/ab	a/b
	Anderson, Indiana	...	...	...	...	a
1591(5).1	Morrilton, Ark.	...	...	...	...	a
1591(10).1b	Morrilton, Ark.	...	...	...	-/ab	a
1586(8).3a	Morrilton, Ark.	...	...	...	...	a/b
1879.6j	Williamston, N.Carol.	...	...	...	...	a
1880.6c	Richmond, Va.	-/ab	...	...	...	a
1897.18	Allegeny Park, N.Y.	...	...	...	...	a/b
1882.6	Millersburg, Penn.	...	...	...	-/ab	b
1882.6b	Millersburg, Penn.	...	...	...	-/ab	b
1893.10	South Hero, Vt.	...	...	...	-/ab	a/b
1899.6a	Chagrin Falls, Ohio	...	...	...	-/ab	a/b
1901.5a	Jackson, Mich.	...	...	...	...	a
1901.5b	Jackson, Mich.	...	...	...	...	a/b
1901.5d	Jackson, Mich.	...	...	...	...	a/b

TABLE 1—Continued

Inversion Variations in *Drosophila americana americana*

Strain No.	Locality	X	Western Strains 2	3	4	5
1773.4c	Chadron, Nebr.	-/d	.....	.....	ab;-/c	b
1773.4e	Chadron, Nebr.	d	-/b	.....	ab	b
1773.4f	Chadron, Nebr.	.....	-/b	.....	ab	b
1773.4i	Chadron, Nebr.	-/d	-/b	.....	a	b
1773.4j	Chadron, Nebr.	.....	.....	.....	ab	b
1773.4l	Chadron, Nebr.	.....	-/b	.....	ab	b
1760.8a	Poplar, Mont.	.....	.....	.....	-/ab	b
1760.8b	Poplar, Mont.	.....	.....	.....	a	b
1760.8c	Poplar, Mont.	.....	.....	.....	ab	b
1760.8d	Poplar, Mont.	.....	.....	.....	a	b
1760.8f	Poplar, Mont.	.....	-/b;-/c	-/a	-/ab	b
1760.8g	Poplar, Mont.	.....	-/b	.....	ab	b
1760.8h	Poplar, Mont.	.....	.....	.....	ab	b
1760.8i	Poplar, Mont.	.....	-/b	.....	ab	b
1760.8j	Poplar, Mont.	.....	.....	-/a	ab	b
1760.8k	Poplar, Mont.	.....	.....	.....	-/ab	b
1760.8l	Poplar, Mont.	.....	.....	.....	-/ab	b
1760.8m	Poplar, Mont.	.....	b	.....	-/a	b
1760.8q	Poplar, Mont.	.....	.....	.....	-/ab;-/c	b
1760.8r	Poplar, Mont.	.....	b	.....	ab	b
1760.8s	Poplar, Mont.	.....	-/b	.....	ab	b
1760.8t	Poplar, Mont.	.....	-/b;-/c	.....	-/ab	b
1760.8u	Poplar, Mont.	.....	.....	.....	-/ab	b
1760.8v	Poplar, Mont.	.....	-/b	.....	-/ab	b
1760.8w	Poplar, Mont.	.....	.....	.....	a;-/b	b
1760.8x	Poplar, Mont.	.....	.....	.....	ab	b
1760.8y	Poplar, Mont.	.....	.....	.....	abc	b
1760.8z	Poplar, Mont.	.....	-/b	.....	ab	b
1761.9a	Chinook, Mont.	.....	-/b	.....	ab	b
1761.9s	Chinook, Mont.	.....	-/b	.....	a	b
2067.1b	Chadron, Nebr.	.....	.....	.....	ab	b
2067.1e	Chadron, Nebr.	.....	.....	.....	a;-/b	b
2067.1h	Chadron, Nebr.	.....	-/b	.....	ab	b

Probably *americana* is the most variable form in this complex (Table 1). The strains already examined by Warters are also included in the table with our system of nomenclature of inversions. With the exception of the dot-like chromosome, every chromosome has at least two kinds of gene alignments. In the X-chromosome, a few eastern strains may have the *texana* type chromosome, i.e., with Xab instead of Xabs. In a number of strains from Chadron, Nebraska, the small inversion Xd is present. In some strains collected from Montana and Nebraska, the second chromosome exhibits a subterminal inversion identical with the inversion 2b of *novamexicana*, but none of the eastern strains contains it. Further, a very small proportion of the western strains also have the *novamexicana* inversion 2c.

Most strains have the third chromosome identical with the standard, i.e., the *virilis* arrangement. However, two of the western strains possess the *novamexicana* inversion 3a. The fourth chromosome is extremely variable, sequences 4, 4a, 4ab, and 4abc may all be present in one or the other strains. The western strains have more of the 4a or 4ab type, while the eastern strains have more of the *virilis* type. Inversion 4c is present only in the western strains.

The western strains show a consistent gene arrangement on chromosome 5, i.e., the *novamexicana* type 5, with inversion 5b but without 5a. Relatively few of the eastern strains are homozygous for this type of sequence, most of them being heterozygous for 5a and 5b, or homozygous for 5a.

The basic inversion formula for *americana* is thus Xabc, 2a, 3, 4, 5a (or 5b), 6.

IV. *Drosophila americana texana* Patterson, Stone and Griffen.

The test strain (New Orleans) of *texana* has all the chromosomes identical with the test strain of *americana* (Anderson) except the X, which is Xab instead of Xabc. However, some other strains may have Xc. No variations have been found in the second and the third chromosomes, while the fourth and the fifth chromosomes are occasionally variable.

TABLE 2

Inversion Variations in *Drosophila americana texana*
Basic formula: Xab, 2a, 3, 4, 5(a or b);
, chromosomes identical to the basic formula;
/, heterozygous for the respective inversion;
*, data from Warters, 1944.

Strain No.	Locality	X	2	3	4	5
*84.7	Georgetown, Tex.					a/b
*821.12b	Georgetown, Tex.					a
*821.12c	Georgetown, Tex.	c			-/ab	b
*825.13c	Anderson, Tex.				-/ab	a
*841.10	Newton, Tex.					a
*849.11	Belton, Tex.	-/c			-/ab	a/b
*1265.12a	Cross Lake, La.					a
*	Lake Isala, Fla.					a
*1148.9	Lake McKethan, Fla.					a
*1173.6a	Okefenokee Swp., Ga.					a
*1173.6c	Okefenokee Swp., Ga.					a
*1173.6d	Okefenokee Swp., Ga.					a
*1271.13q	Hiwassee Resv., Tenn.	-/c				a
1128.10	New Orleans, La.					a
1878.7	Albermarle, N.Carol.					a
1879.6c	Williamston, N.Carol.					a
1879.6f	Williamston, N.Carol.					a
1879.6h	Williamston, N.Carol.					a
1879.6l	Williamston, N.Carol.	-/c				a
1879.6m	Williamston, N.Carol.					a
1879.6n	Williamston, N.Carol.					a
1880.6a	Richmond, Va.					a
1880.6j	Richmond, Va.					a
1591(4).1	Morrilton, Ark.					a
1591(7).1d	Morrilton, Ark.					a
1591(D).1	Morrilton, Ark.					a/b
1592.6a	Caddo Lake, La.					a
1593(B).6b	Caddo Lake, La.					a
1593(B).6c	Caddo Lake, La.					a
1593(B).6d	Caddo Lake, La.					a
1593(B).6e	Caddo Lake, La.					a
1593(B).6f	Caddo Lake, La.					a
1282.11a	Arkansas Riv., Ark.					a
1282.11b	Arkansas Riv., Ark.					a
1278.14b	Eva, Tenn.					a
195.6	Fort Worth, Tex.					b
1533.1	Alabama					a
1533.2	Alabama					a
2020.1e	Tombigbee Park, Miss.	-/c				a
2007.6j	Tallahassee, Fla.					a

Only a few strains contained the inversions 4ab, and a few contained the *novamexicana* 5, 5b. All these variations were found in the strains from the overlapping zone. Table 2 gives the results of the analysis.

V. *Natural hybrids between americana and texana.*

Natural hybrids between *americana* and *texana* have been reported by Stone and Patterson (1947). Several new strains were proven to be hybrids or intercross descendants of *americana* and *texana*. Among the 14 strains collected from Williamston, North Carolina, six are hybrid (strains 1789.6a, b, d, e, g and j), and among the seven strains from Richmond, Virginia, four are hybrid (strains 1880.6d, f, g and i). The autosomal gene arrangements of these hybrids are identical to those of both the Anderson and the New Orleans strains. The X-chromosome shows variation in inversion contents as follows:

North Carolina hybrids:		Virginia hybrids:	
Xab/Xab	3 strains	Xab/Xab	1 strain
Xab/Xabc	3 strains	Xab/Xabc	2 strains
		Xabc/Xabc	1 strain

Drosophila littoralis Meigen

Morphologically, *D. littoralis* resembles the members of the montana complex. Cytologically, it has a pair of large V's, a pair of J's, two pairs of rods and a pair of dots. *Drosophila imeretensis* has the same metaphase configuration. The V-shaped chromosome is proven by salivary preparation to be the result of fusion between chromosomes 3 and 4. The J-shaped chromosome corresponds to the second chromosome of *virilis*, but with the centromere located near the middle of the euchromatic part.

Drosophila littoralis (stock 2000.1, from Merlingen, Switzerland) will produce hybrids with *virilis* when *virilis* is used as the female parent. VL hybrid females are slightly fertile to *texana* and *montana* males. Much of our information is from these outcross hybrids.

The X-chromosome. The *virilis/littoralis* hybrids show a complex figure of the X-element, containing about six or seven inversions overlapping one another, and we have not traced out the sequencial changes. A symbol X"Li" is used to denote the X-chromosome of *littoralis*, implying all the unknown inversions.

The second chromosome. In *littoralis* the second chromosome is one of the most important elements in our information on chromosome evolution in this group. First, the centromere is metacentric. Second, there is a big, dark staining, heterochromatin-like block near the tip of the shorter arm, the origin of which is not known.

The photomicrograph (Fig. 1) shows the second chromosome of the *virilis/littoralis* hybrids. Starting from the longer arm of *littoralis*, the two species have a homologous section of considerable length from the

tip 2Aa to the place between 2Fj and 2Fk, where a short inversion begins that ends between 2Ig and 2Ih. This is the inversion 2i. A short paired section follows. The two chromosomes separate at the distal break of the *texana* inversion 2a, and an inversion loop is observed up to the proximal limit of 2a, except that the centromere of *littoralis* is in the 2a

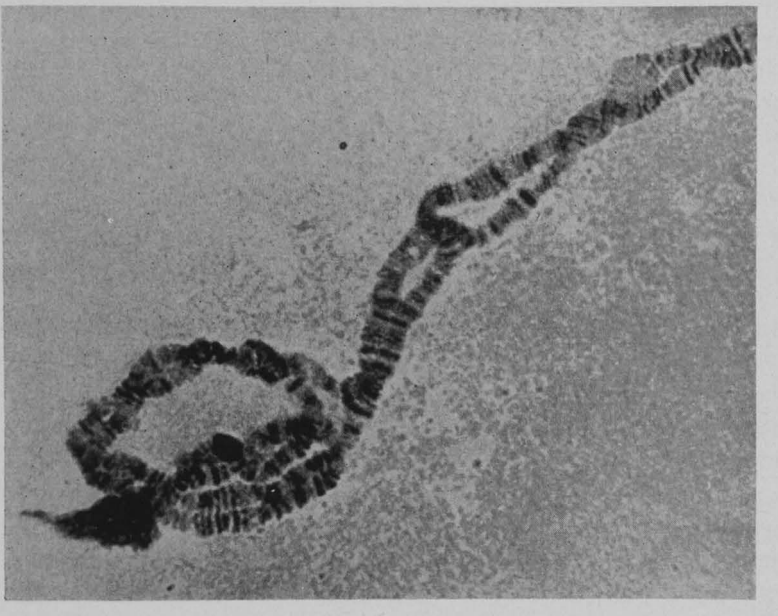


FIG. 1. Photomicrograph of the second chromosome of the *virilis/littoralis* hybrid, showing inversions 2a, d, e and i.

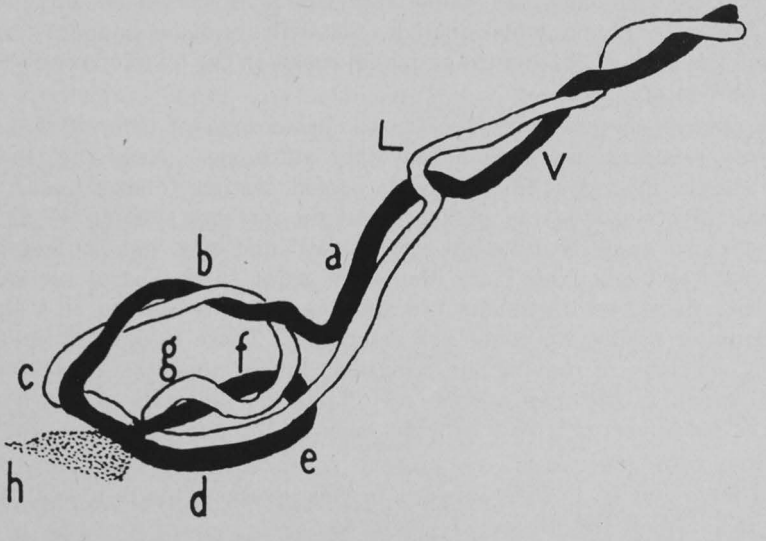


FIG. 2. Diagram of fig. 1 showing the gene sequences.

inversion loop. Had *littoralis* not had the location of the centromere changed, the picture would be a simple 2a inversion. Thus the second chromosome of *littoralis* differs from that of *texana* only in the following respects.

1) a small inversion 2i, and

2) the centromere position is located somewhere near 2Po, instead of at the end. It differs from *virilis* in one more step, the inversion 2a. If we label the section of the chromosomes as in Fig. 2, we get the gene sequences as

virilis	a b c d e f g . h
littoralis	a e d . c b f g h

where the black dots represent the centromeres and h is a small piece of chromatin at the very end.

Starting from *virilis*, a first inversion reverses the order bcde to
a e d c b f g . h

which is the *texana* inversion 2a. The second inversion inverts the section cbfg into

a e d g f b c . h (inversion 2d)

and the third inversion, a pericentric one, brings the centromere to the middle,

a e d . c b f g h (inversion 2e).

Therefore, the second chromosome of *littoralis* has the inversion 2adei as compared with that of *virilis*. It is not possible to determine whether 2d or 2e occurred first.

The third chromosome. The stock is heterozygous for an inversion on the third chromosome. If this inversion (3i) is not present, the *virilis/littoralis* hybrids show the simple subterminal inversion 3h only. Inversion 3i is a large one, which includes 3h, with its distal boundary a little beyond the limit of 3h, and the proximal break on the middle lower portion, map section 3p.

The fourth chromosome. The fourth chromosome of *littoralis* has more complex rearrangements than the other autosomes. Near the tip there is a simple inversion 4d. A short, paired section follows and a long, median inversion (4e) is present between the map section 4I and 4Q. Its proximal break is probably between 4Qf and 4Qg, but another inversion (4f) extends from near the same point to the basal section 4Y. Whether these two inversions (4e and 4f) are of a tandem or a slightly overlapping nature we could not determine. There is another inversion which overlaps 4f having its proximal break near the base and the distal break in the map section 4X. Thus the majority of the sections that 4f has inverted is now brought back by the new inversion, 4m.

These four inversions are present consistently in the stock, but a small inversion 4n is heterozygous in this strain. This is a small inversion included in the longer inversion 4e.

The fifth chromosome. Three inversions are present in the fifth chromosome: a long, almost terminal inversion 5j, and two long inversions near the base. One of the basal series (5i) is included in the other (5h). As can be seen from the diagram (Fig. 11), the two inversions have their proximal breaks very close to each other, and the distal breaks apart. Thus in the *virilis/littoralis* hybrids, a long section can often be observed bulging out near the base of the *littoralis* chromosome and the same section is looped out in the portion in the *virilis* chromosome.

In summary, if we put parentheses for the heterozygous inversions, the inversion formula for the *littoralis* stock 2000.1 would be X"Li," 2adei, 3h(i), 4defm(n), 5hij, 6.

THE MONTANA COMPLEX

As has been stated before, the montana complex consists of four forms, namely, *D. montana, D. montana* yampa strains, *D. montana* superior strains, and *D. lacicola.* These forms differ morphologically as well as cytologically. The basic metaphase configuration is the same in all the forms, being four pairs of rods, a pair of dots, and a pair of J's which corresponds to the second chromosome of *virilis,* but with the centromere located near the mildle of the euchromatic part.

I. *Drosophila montana* Patterson and Wheeler.

Our test stock, 1218.8d from Cottonwood Canyon, Utah, is the only stock left from the old strains that Warters used for her analysis, but it helps us a great deal in understanding the old data. It is a vigorous stock and the females are quite acceptable to males of other strains.

Cytological examinations have been made of *montana* strains from the following localities: Mather, Lake Tahoe and Truckee, California; Reno, Nevada; Little Salmon River, Idaho; Grand Teton National Park, Yellowstone National Park and Lander, Wyoming; the east fork of San Juan River, Archuleta County, Colorado; and Raton, New Mexico.

Drosophila montana and *virilis* can produce some hybrids, and *texana* females and *montana* males will give a fair yield of hybrids (Patterson and Griffen, 1944). Both types of hybrids show a good many rearrangements in the salivary gland chromosomes, and some of them are extremely complicated. Using the *virilis* chromosome map as standard, a brief description of the rearrangements of the test strain of *montana* are as follows:

The X-chromosome. The rearrangements in the X-chromosome have proven to be too complex for analysis in both the *virilis* and the *texana* hybrids. The homologs seldom pair. For practical purposes, we use the symbol XM to represent the X-chromosome of *montana.*

The second chromosome. It is known from Griffen's analysis that the centromere position of the second chromosome of *montana* is metacentric. A comparison of the *virilis/montana* hybrids (Fig. 3) and *texana/montana*

hybrids clearly reveals that *montana* has the same basic changes as *littoralis*, i.e., it possesses the inversion 2a, and the same location for the centromere, near 2Po. A direct proof has later been established by Prof. Patterson who obtained VL × M offspring, among which some have the combination of the *montana* and *littoralis* second chromosomes. The two chromosomes pair perfectly in the vicinity of the centromeres. However, *montana* does not have the *littoralis* distal inversion 2n; instead, it has two small additional inversions one after another, located at that region. These two inversions, 2j and 2k, are so close to each other that the proximal break of 2j and the distal break of 2k look to be identical. In the *littoralis-montana* combination, this region shows three inversions as expected.

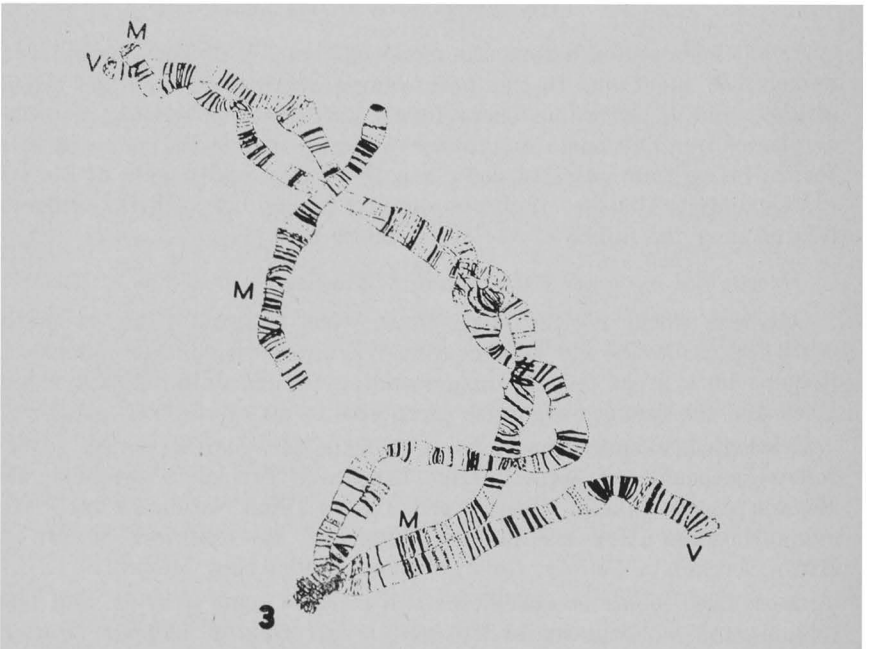


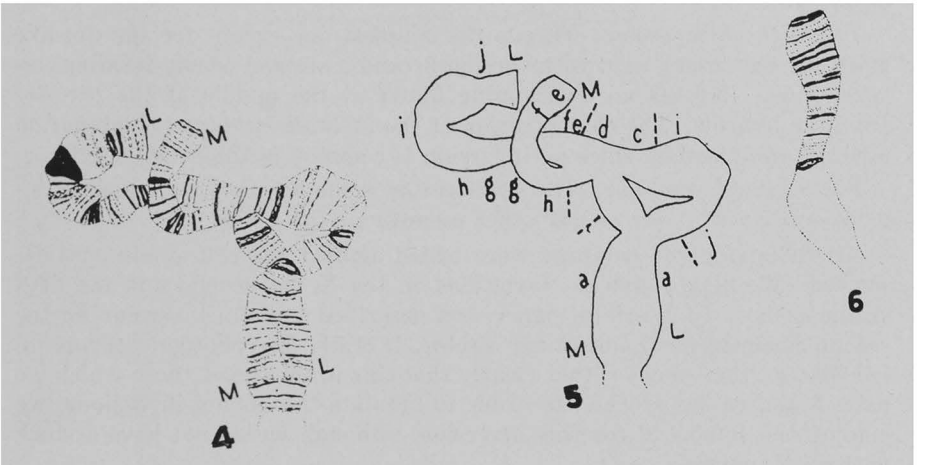
FIG. 3. Camera-lucida drawing of the second chromosome of the *virilis/montana* hybrid; the right arm of *montana* chromosome is broken.

The shorter arm of the second chromosome of *montana* has undergone further changes. The order of the sections 2U through 2Z is completely inverted, with the section U as the tip of that arm. But a simple inversion would not produce the *montana* figure, because the section 2Kj to 2Le which is adjacent to the section U in *texana* and *littoralis* is now inserted into the place just before 2Wa. The *littoralis-montana* combination (Figs. 4 and 5) enables us to make a series of three hypothetical steps of inversions to change the *littoralis* picture to the *montana* sequence, not including the big block of heterochromatin of *littoralis*. If as indicated in the diagram in Fig. 5, the *littoralis* and the *montana* chromo-

some sequences are given as a c d e f g h i j and a j i c d h g f e, respectively, the three steps would be as follows:

1) a c d *e f g h i j* ——> a c d j i h g f e (inversion 2f)
2) a c *d j* i h g f e ——> a j d c i h g f e (inversion 2g)
3) a j *d c i* h g f e ——> a j i c d h g f e (inversion 2h)

If this is true, this section from 2Le through the tip of the chromosome has undergone three successive inversions, 2f, 2g and 2h, respectively, to produce the *montana* sequence. Adding these inversions to the basic 2ade inversions, our test strain of *montana* has the second chromosome formula: 2adefghjk.



FIGS. 4–6. Fig. 4—Camera-lucida drawing of the right end of the second chromosome of the *littoralis-montana* combination; Fig. 5—diagram of fig. 4, showing gene sequences; Fig. 6—Camera-lucida drawing of the fifth chromosome of the *montana/lacicola* hybrid, showing a single band variation.

The third chromosome. The third chromosome of *montana* does not have any of the *littoralis* inversions, but two rather long inversions as compared with *virilis*. The first one (3b) is submedian and the second one (3c) overlaps it. The *littoralis-montana* combination (especially when both 3h and 3i are present) show a complicated configuration.

The fourth chromosome. Drosophila montana has the same basic inversions of the fourth chromosome as *littoralis*, i.e., 4def. However, the terminal portion of this chromosome in *montana* has an additional inversion overlapping the basic 4d. In *virilis/montana* hybrids, the terminal segment clearly shows a typical overlapping figure, and in *littoralis-montana* combinations, only a simple, nearly terminal inversion is found. This inversion is designated as 4h. The basal portion is more complicated and more interesting. Superficially, the *virilis/montana* hybrids show exactly the same figure as the *virilis/littoralis* hybrids in this region. But the *littoralis-montana* combination reveals the difference, i.e., although the two chromosomes pair very well, there are a few bands near the base in

littoralis which *montana* does not have, and on the contrary, *montana* has a few bands at the middle lower portion which *littoralis* lacks. Careful check and crucial comparison lead to the conclusion that *montana* does not have the inversion 4m, instead, it has another inversion 4g which also overlaps 4f and has the breakage points so close to those of 4m that they are easily overlooked. They both have the distal break in map section X which was already inverted by 4f; but *montana* has the breaks between 4Xc-b and 4Yl-m, while in *littoralis* the breaks are between 4Xh-g and 4Yn-Za. Thus the short section Xh-Xb is left in the middle lower portion in the 4th chromosome of *montana*, and is brought back to near the base in *littoralis*. In other words, the test strain of *montana* has its fourth chromosome formula as 4defgh.

The fifth chromosome. This is the simplest one except for the dot-like sixth. It has a long central inversion 5c and a simple, nearly terminal inversion 5d. It gives an overlapping figure at the middle of the *texana/montana* hybrids. The configuration of the *littoralis-montana* combination again is complicated, since no inversion is common to these two species.

The sixth chromosome. The sixth chromosome has the same gene alignment as that of *virilis* or any other member of the group.

All the strains of *montana* were tested against the test strain just described. We have found no variations on the X, the second and the fifth chromosomes. In Warters' paper, she described a distal inversion on the second chromosome in one of her strains, 1211.51. The photomicrograph in her dissertation shows rather clearly that this is not one of those which we have found in our stocks. In order to tabulate the known inversions, we employ the symbol 2*l* for this inversion, although we do not have a stock with it to analyze.

Table 3 shows the analytical data of the available strains tested (39 strains). The only inversion found in the third chromosome is the "upper" inversion of Warters, which is present in some of the strains from Wyoming and Idaho.

The fourth chromosome may show four kinds of inversions against the test strain. In the test procedure, strains showing 4h inversion to the test strain do not have this inversion, and strains showing 4g lack the inversion. The other two inversions are not found in the test strain. One of them is 4i, which is a small inversion included in 4e. It is probably the same as the "median" inversion of Warters. The last one is designated as 4o, which overlaps the e, f, and i inversions. The inversion 4o has never been found in the same chromosome with the inversion 4g, but is always associated with 4i.

In her paper Warters described four inversions: the terminal (probably 4h), the median (4i), the basal, and a distal inversion. Whether she found the inversion 4o but included it in the category as "basal" (4g) we do not know, but the distal inversion is distinct in her photomicrograph (p. 147), as another inversion we have not found. In our system of nomenclature, this inversion is known as 4*l*.

It is clear from Table 3 that none of the California or Nevada strains contain the inversion 4g, and very few of them have 4h. The inversion 4i, on the other hand, is present in these strains in a rather significant frequency. The Idaho and Wyoming strains are more or less similar to the California and Nevada strains with the additional 3d and 4o inversions, though the Lander strains contain 4h exclusively.

The strains from Colorado and New Mexico are all identical to the standard, and are all homozygous.

TABLE 3

Inversion Variations in *Drosophila montana*

Basic formula: XM, 2adefghjk, 3bc, 4def, 5cd;
....., chromosomes identical to the basic formula;
/, heterozygous for the respective inversion.

Strain No.	Locality	X	2	3	4	5
1218.8d	Cottonwood Canyon, Utah	.....	.....	.....	gh	.....
	Mather, Calif.	.....	.....	.....	–/i	.....
1862.2a	Lake Tahoe, Calif.	.....	.....	.....	i	.....
1862.2b	Lake Tahoe, Calif.	.....	.....	.....	i	.....
1862.2c	Lake Tahoe, Calif.	.....	.....	.....	i	.....
1942.6a	Reno, Nev.	.....	.....	.....	–/i	.....
1942.6b	Reno, Nev.	.....	.....	.....	i	.....
1942.6c	Reno, Nev.	.....	.....	.....	i	.....
1942.6d	Reno, Nev.	.....	.....	.....	–/i	.....
1942.6e	Reno, Nev.	.....	.....	.....	–/i	.....
1942.6f	Reno, Nev.	.....	.....	.....	i	.....
1942.6g	Reno, Nev.	.....	.....	.....	–/i	.....
1942.6h	Reno, Nev.	.....	.....	.....	.....	.....
1942.6i	Reno, Nev.	.....	.....	.....	i	.....
1942.6j	Reno, Nev.	.....	.....	.....	.....	.....
1942.6k	Reno, Nev.	.....	.....	.....	.....	.....
1942.6l	Reno, Nev.	.....	.....	.....	.....	.....
1942.6m	Reno, Nev.	.....	.....	.....	i	.....
1942.6n	Reno, Nev.	.....	.....	.....	i	.....
1942.6o	Reno, Nev.	.....	.....	.....	–/h	.....
1942.6p	Reno, Nev.	.....	.....	.....	i	.....
1943.6a	Truckee, Calif.	.....	.....	.....	–/h;–/i	.....
1972.1	California	.....	.....	.....	i	.....
1956.5a	San Juan Riv., Colo.	.....	.....	.....	gh	.....
1956.5b	San Juan Riv., Colo.	.....	.....	.....	gh	.....
1956.5c	San Juan Riv., Colo.	.....	.....	.....	gh	.....
1956.5d	San Juan Riv., Colo.	.....	.....	.....	gh	.....
1956.5e	San Juan Riv., Colo.	.....	.....	.....	gh	.....
1956.5f	San Juan Riv., Colo.	.....	.....	.....	gh	.....
1767.5a	Little Salmon Riv., Ida.	.....	.....	.....	i;–/g;–/h	.....
1767.5b	Little Salmon Riv., Ida.	.....	.....	–/d	i	.....
1769.1a	Grand Teton, Wyo.	.....	.....	–/d	hi	.....
1769.1b	Grand Teton, Wyo.	.....	.....	–/d	.....	.....
2064.2a	Lander, Wyo.	.....	.....	.....	h;–/g;–/i	.....
2064.2b	Lander, Wyo.	.....	.....	.....	hi;–/o	.....
2064.2c	Lander, Wyo.	.....	.....	.....	hi;–/o	.....
2064.2d	Lander, Wyo.	.....	.....	–/d	hi;–/o	.....
2064.2e	Lander, Wyo.	.....	.....	.....	hi;–/o	.....
2064.2f	Lander, Wyo.	.....	.....	.....	h;–/g;–/i	.....
2072.4	Raton, New Mexico	.....	.....	.....	gh	.....

II. *Drosophila montana* yampa strains.

Warters found a number of *montana* strains, including 1218.8d, giving "subterminal, median and included" inversions when crossed to her first standard, 1210.98. However, we have found no inversion variations in the

X of all the strains of *montana* thus far studied. On the other hand, all the yampa strains give such inversions when crossed to *montana*. In fact, the two median inversions are not "included," as judged from our preparations and Warters' photomicrograph, but overlapping inversions. Therefore, presumably the first standard of Warters was a yampa stock, and her second standard, 1212.5c from Madison River, was a true *montana*. A further proof was found in the permanant collection record where it states that 1210.98 was a pair mating strain by light × light. In other words, Warters' *montana* strains contained both forms.

The yampa strains give more hybrids with *montana* when yampa is used as the female parent. In our collection data, yampa has been identified from the following localities: Emmett, Carey and Chester, Idaho; Yellowstone National Park, Wyoming; Hamilton and Carbondale, Colorado; and Chama, New Mexico. We have not found the two forms living together in the same locality, but according to Warters' data, they apparently do so in the Yellowstone and Grand Teton regions.

Comparing our test strain (1953.8a, from Carbondale, Colorado) with 1218.8d of *montana*, it shows the inversions on the X-chromosome just mentioned, viz., a simple, small, subterminal inversion, and two inversions overlapped on the middle portion. Since we do not know what arrangement the *montana* X has, we are not able to plot the breakages of the yampa inversions either. Arbitrarily we call the two overlapping inversions Xf and Xg, and the subterminal one as Xe, but they are not based on the *virilis* standard map. Moreover, whether these inversions are closer to, or farther from, the *virilis* gene sequence than the *montana* X is also a question.

The second chromosome of our test strain is identical to that of *montana*. The third chromosome contains one additional inversion (3e) added to the standard *montana* arrangement, 3bc, and this inversion 3e probably corresponds to the "lower" inversion of Warters. The fourth chromosome gives a rather complex figure in hybrids between the test strain of yampa and the test strain of *montana*. Actually there are three overlapping inversions. The distal section of 4 is homologous for a considerable distance, indicating that the inversions 4d, 4e, and 4h are all present in yampa. The three inversions on the middle lower portion are identified as the inversion 4g with two new inversions: a long, nearly basal inversion 4k and a short, median one 4j. The presence of the 4g inversion means that our test strain of yampa does not contain it. The proof is provided by the hybrid with the montana strain 1942.6j, which lacks the 4g inversion. The figure is much simpler, with two overlapping inversions, a basal longer one (4k) and a median small one (4j). Therefore, the fourth chromosome of the test strain of yampa has the formula 4defgjk.

The fifth chromosome is again simple. It has a rather large simple median inversion (5e) when compared with *montana*, or in other words, it has the formula 5cde. This inversion is very likely the "median" of Warters. As usual there is no variation in the sixth chromosome.

All the strains of yampa have the same X, third and fifth chromosomes. There may be one inversion on the longer arm of the second, which is

nearly terminal. This inversion, 2n, is present in some of the strains in the heterozygous state. The fourth chromosome may be heterozygous for or lack the inversion 4k.

The simplest formula for yampa thus would be XMefg, 2adefghjk, 3bce, 4defhj, 5cde, 6. Table 4 shows the results of the strains tested on the basic formula.

TABLE 4

Inversion Variations in *Drosophila montana* yampa strains

Basic formula: XMefg, 2adefghjk, 3bce, 4defhj, 5cde;
, chromosomes identical to the basic formula;
/, heterozygous for the respective inversion.

Strain No.	Locality	X	2	3	4	5
1950.1b	Chester, Ida.	,	,	,	–/k	,
1950.1c	Chester, Ida.	,	–/n	,	k	,
1951.1a	Hamilton, Colo.	,	–/n	,	–/k	,
1951.1b	Hamilton, Colo.	,	–/n	,	k	,
1951.1g	Hamilton, Colo.	,	,	,	,	,
1951.1h	Hamilton, Colo.	,	,	,	–/k	,
1953.8	Carbondale, Colo.	,	,	,	k	,
1953.8a	Carbondale, Colo.	,	,	,	k	,

It is difficult to transcribe Warters' data into our system. First of all, her two tables (5 and 6) are not in agreement, perhaps due to heterozygous stocks, so we do not know which one should be followed. Second, since the two forms occur together, and the strains were established from pair matings after they had been brought back from the field, some of them may have been crosses between the two forms. Third, the inversions 4g, 4k and 4o could very easily be put together into the category "basal." As a result we have to give up the fourth chromosome while computing the old data, thus losing a good deal of information. However, some of Warters' stocks may still be determined as:

montanas: 1210.100, 1210.122, 1211.59, 1211.65, 1211.66, 1211.69, 1212.5f; yampas: 1210.98, 1210.87, 1210.111.

III. *Drosophila montana* superior strains.

This form was first identified in 1949 at Chester, Idaho and Hamilton, Colorado, occurring together with yampa. Since then further tests have shown that the only strain still in stock from Lake Croix, Wisconsin, 1755.4e, has been proven to be superior. Second, among the stocks collected and sent to this laboratory by Dr. H. T. Spieth from Itasca Park, Minnesota, labelled as *lacicola,* all except one appear to be superior. The exceptional stock, 2077.4g, is a true *lacicola.* The distribution of superior seems to cover both the east and west sides of the main Rocky Mountains, extending eastwards to the Great Lake region in Minnesota, where it coexists with *lacicola,* and westwards to Idaho and western Colorado, where it meets yampa. Whether the distribution of superior overlaps that of *montana* anywhere is not known, but this seems possible, at least geographically if not ecologically.

Judging by the inversions reported, we are reasonably sure that there was no superior stock in Warters' analysis.

The superior strains are cross sterile to both *lacicola* and yampa, but give a few hybrids with *montana*, if superior is used as female parent, and large number of mass matings are made. A comparison was made between the chromosomes of our test strain of superior (1950.1h, from Chester, Idaho) and the test strain of *montana*. The X-chromosome shows two independent inversions, the median Xh, and the small, subbasal Xi. Again these symbols are not based on the *virilis* map, but on the *montana* X, XM.

The second and the third chromosomes have no consistent inversion differences when compared to those of *montana*. The fourth chromosome shows the 4g inversion, indicating the lack of 4g in the test strain of superior. The fifth chromosome has two small independent inversions, one median and one distal. The two inversions on 5 are symbolized as 5f and 5g, respectively. Inversion 5f is actually included in 5c, but 5g is independent of both 5c and 5d.

Among the available strains no variations have been observed in the X and the fourth chromosomes. There is one inversion on the right, shorter arm of the second, 2m, found in the Wisconsin strain only. It is a rather large inversion which almost turns the whole right arm around. It has one break in the centromeric heterochromatin and the other break not far from the tip. The third chromosome may have two kinds of additional inversions: a subterminal, short inversion 3g and a large, basal inversion 3f. Inversion 3g is independent of both 3b and 3c, but 3f overlaps 3b and 3c on the distal side and has the proximal break in the heterochromatin near the centromere. Inversion 4f is present in all the superior strains, but 5g may be absent or in the heterozygous state.

With the exception of 2m, the other inversions are widely distributed. For instance, 3f is very common in Minnesota strains and is also homozy-

TABLE 5

Inversion Variations in *Drosophila montana* superior strains

Basic formula: XMhi, 2adefghjk, 3bc, 4defh, 5cdf;
, chromosomes identical to the basic formula;
/, heterozygous for the respective inversion.

Strain No.	Locality	X	2	3	4	5
1950.1h	Chester, Ida.					g
1951.1y	Hamilton, Colo.			g		
1755.4e	Lake Croix, Wis.		m	f		g
2077.4b	Itasca Park, Minn.			f		g
2077.4c	Itasca Park, Minn.			f		g
2077.4d	Itasca Park, Minn.			f		−/g
2077.4e	Itasca Park, Minn.			f		g
2077.4f	Itasca Park, Minn.			f		g
2077.4h	Itasca Park, Minn.			f		−/g
2077.4l	Itasca Park, Minn.			−/f		g
2077.5a	Itasca Park, Minn.			f		−/g
2077.5b	Itasca Park, Minn.			−/f;−/g		g
2077.5c	Itasca Park, Minn.			f		g
2077.5d	Itasca Park, Minn.			−/f		g

gous in the Wisconsin strain. Inversion 3g and 5f have been found in both the Minnesota and the Colorado strains. Table 5 presents the result of chromosome analysis.

IV. *Drosophila lacicola* Patterson.

So far *Drosophila lacicola* has been investigated by me only from the state of Minnesota. Superficially it looks very much like superior except the body is still shorter and blacker with some minor difference, but crossing between these two species fails completely no matter which strain is used. However, *lacicola* gives a moderate yield of hybrids with *montana* when *montana* is used as the female parent and the *virilis-lacicola* cross gives a few offspring (Patterson and Griffen, 1944).

Our test strain (1360.1, from Fairbanks, Minnesota) is not a homozygous one, since it contains three inversions in the heterozygous, one on the second and two on the fifth. A comparison of the test strain to *montana* (1218.8d) shows the following differences:

The X-chromosome. The X-chromosome of *montana/lacicola* hybrids shows a highly complicated system of changes; and *virilis/lacicola* hybrids have the two X-chromosomes completely separated. This indicates that the *lacicola* X probably came from the *montana* X and has further changed greatly during the history of *lacicola.* XM"Lc" is used as the symbol for the *lacicola* X-chromosome.

The second chromosome. There are two inversions, one included in the other, located near the tip of the long arm of the second chromosome. Other portions, including the centromere, are homologous all the way. The two inversions near the tip are 2o and 2p, the latter being included in the former. At the tip of the shorter arm, another inversion 2q is present in the heterozygous state. It is a small inversion, almost terminal.

The third chromosome. There are two additional inversions on the basic *montana* 3bc possessed by *lacicola.* Inversion 3j is a basal one which overlaps the basic 3b and 3c, while inversion 3k overlaps 3j again, with the proximal break just before the disk-like region near the base. No variations are found in this stock.

The fourth chromosome. The stock does not contain the inversion 4h and 4g. In addition to the basic 4def, it has two other inversions, 4p and 4q. These inversions are very perplexing, because their proximal breaks are so close together that only a few faint bands are in between. Inversion 4p has the proximal break in the map section 4X (already inverted by 4f) and the distal break just below the distal break of 4e, so it is actually a rather small inversion. The inversion 4q has the proximal break right below that of 4p, and the distal break in the map section 4P, which has been inverted by 4e. In *montana/laciola* hybrids, inversions 4g, 5p, 4q and 4h are all present and thus make a complex configuration.

The fifth chromosome. Only one of the three inversions found in the 5 is constant for this stock, i.e., the basal inversion 5k. The other two inversions are present in the stock in the heterozygous condition. These are

5*l*, a small inversion included in the basic inversion 5c, and 5m, a small independent distal inversion.

Near the terminal portion of the fifth chromosome, the *lacicola* strain shows a single band difference (Fig. 6), i.e., a band is missed in *lacicola* and the section is always unpaired in the *montana/laciola* hybrids.

Putting the heterozygous inversions in parentheses, we can summarize the inversion formula of this stock as XM"Lc," 2adefghjkop(q), 3bcjk, 4defpq, 5cdk(l, m), 6.

Three other stocks were available. Since none of the *lacicola* strains is homozygous, each was tested against *montana* (1218.8d) separately. A brief account of the inversion variations is given below:

Strain 1360.2, Fairbanks, Minnesota. This is somewhat like 1360.1 with one outstanding feature, i.e., it is heterozygous for the inversion 4h. This would mean that the fourth chromosome, like that of *montana*, may or may not possess the 4h inversion. The fifth chromosome does not have the inversion 5*l* and 5m, but 5k is still present and is homozygous.

Strain 1756.2b, Fenske Lake, Minnesota. This is also heterozygous for 4h. The fifth chromosome has no k, *l*, or m, but another inversion near the base, 5n, in homozygous state. Hybrids between this and any other strain containing 5k show an overlapping figure on the basal portion of 5.

Strain 2077.4g, Itasca Park, Minnesota. The outstanding feature of this stock is the heterozygosity for the inversion 2o and 2p. The two included inversions are either present or absent together. In addition, a new inversion 2r is found in the heterozygous state located near the base of the shorter arm of the second chromosome.

All the four inversions on the 2nd and 5th chromosomes are thus not consistent for *lacicola*. Leaving out the X-chromosome about which we know almost nothing, the basic pattern of the other chromosomes can be tentatively formulated as 2adefghjk, 3bcjk, 4defpq, 5cd and 6. Using this basic formula, the variations of the *lacicola* stocks are shown in Table 6 in more detail.

TABLE 6

Inversion Variations in *Drosophila lacicola*

Basic formula: XM"Lc", 2adefghjk, 3bcjk, 4defpq, 5cd;
, chromosomes identical to the basic formula;
/, heterozygous for the respective inversion.

Strain No.	Locality	X	2	3	4	5
1360.1	Fairbanks, Minn.	M"Lc"	op;-/q	.....	.....	k;-/l;-/m
1360.2	Fairbanks, Minn.	M"Lc"?	op;-/q	.....	.....	k
1756.2b	Fenske Lake, Minn.	M"Lc"?	op;-/q	.....	.....	n
2077.4g	Itasca Park, Minn.	M"Lc"?	-/op;-/q;-/r	.....	.....	k

DISCUSSION

The present data on the cytological species differences in the *virilis* group serves as an example illustrating the evolutionary trend for related species and subspecies. In plant material, where polyploidy and polysomy are common, the problem of species relationships could be partially solved by cyto-

logical observations on the meiotic division of the hybrids. In Oenothera and other plants with extensive translocations, species relationships can also be partially traced by detecting the ring formation in the hybrids. In Drosophila, these methods employed in plants are not useful.

Using the salivary analysis technique, some cases in the Dipterous insects have been analyzed more fully. For instance, *D. melanogaster* and *D. simulans* are known to differ in one major inversion and a number of minute chromosomal changes (Horton, 1939). None of the other species in the melanogaster group has been known to be hybridizable with any other member in the same group. In the new world section of the obscura group, namely, *pseudoobscura, persimilis* and *miranda,* the chromosomal relationships can be largely traced by the salivary gland chromosome technique, especially between *pseudoobscura* and *persimilis* (Tan, 1935; Dobzhansky and Tan, 1936). *D. miranda* has undergone more profound changes, especially in chromosomes XL, 3, 4, and 5. The chromosomal changes are mainly inversions, with a few translocations, and a number of minor, unmatched sections of the salivary pattern. The relationships of these three species, as revealed by Dobzhansky and Tan's work, would be as follows: *persimilis-pseudoobscura-miranda.* However, different species of Drosophila do not necessarily have extensive chromosomal differences. For instance, in the repleta group, related species such as *D. repleta, D. canapalpa, D. limensis* and *D. malenopalpa* have very little chromosome difference; only a few inversions and a few unmatched heterochromatin sections are encountered in all the four well-isolated forms (Wharton, 1942; Ward and Stone, 1952). But in a species group like virilis, where a number of inversions can be found in the pure species as well as in the hybrids, the analysis permits one to reach some conclusions about the problem of phylogeny.

Practically all the chromosomal rearrangements occurring in wild populations have at least two breakages. If one inversion involves two breaks on a chromosome, the chance of a second inversion having the same breakage points will be extremely low. Thus if we found an identical inversion in two different species with a different sequence in a third, we can logically say that the former two are more closely related. For instance, if species X differs from species Z in two inversions a and b on a certain chromosome, while species Y differs from Z in only inversion b, this would mean the phylogenetic relationship *X a* Y *b Z*. This scheme does not imply which species is more primitive or more highly evolved, unless there is other evidence. If many species and many rearrangements were available, a reasonable phylogenetical relationship could be drawn up by tracing the rearrangement.

In all the species in the *virilis* group analyzed, only two major types of rearrangements were found, viz., inversions and fusions. Minor changes are of a less important role here. So far we have found only one unmatched section with a single band difference between the fifth chromosome of *montana* and the fifth chromosome of *lacicola,* while Dobzhansky and Tan have found many in the *pseudoobscura-miranda* hybrids. Another instance which is analogous to these would be the heterochromatin-like block found

in the second chromosome of *littoralis* which is not present as such in any other species in this group. Translocations apparently have not been involved here, unless we consider fusion as a special type of translocation. Fusions change the metaphase configuration while simple paracentric inversions do not, but as far as the number of the breakages is concerned, the two types of changes are the same, i.e., two breakages and reunion for each. But fusion of two chromosomes is usually accompanied by a loss of a free centromere, and a loss is more easily accomplished than a gain. Therefore it has been generally agreed that in Drosophila the karyotype with five pairs of rods and a pair of dots is the most primitive form (Sturtevant and Novitski, 1941; White, 1945; Wharton, 1943; etc.).

In the virilis group, only *virilis* and *novamexicana* have this type of metaphase chromosome configuration. The rest of the species are, accordingly, derived from this type either by fusion or by pericentric inversion or both. Considering the inversion differences between *novamexicana* and the more highly evolved *americana* and between *virilis* and *americana*, we can readily tell that *novamexicana* is actually closely related to *americana* despite the primitiveness of the metaphase plate. Further, the fact that *novamexicana* females are very difficult to cross with other species while *virilis* females readily accept in hybridization experiments also suggests that *virilis* might be the closest to, if not exactly, the primitive form of the virilis group.

Taking the inversion contents into consideration, we see that all the wild forms show the inversion 2a against *virilis*. If *virilis* were the primitive form, a single inversion 2a might change it into another form, designated in Figure 12 as P_I, from which all the modern wild forms were derived.

Morphologically, physiologically and ecologically *virilis* differs from all the wild forms in many aspects, e.g., the pupa case color, the etherization effect, the food source and other habitats. An alternative suggestion is that the 2a-bearing P_I was the real ancestor and both *virilis* and the wild forms were derived from it. From P_I to virilis, one inversion and some genic changes were required. However, we have no direct evidence as to which alternative is correct.

But it is plausible that P_I gave rise to P_{II} by inversion Xab, and to P_{III} by inversions 4def plus the pericentric inversion on the second, 2de. P_{II} was the ancestor of the americana complex, and had the primitive chromosome type, five pairs of rods and a pair of dots. P_{III} was the ancestor of both *littoralis* and the members in the montana complex.

Let us first consider the changes involved in the americana complex. All the forms in this complex have the X-chromosome inversions a and b, which do not occur in the montana complex or in *littoralis*. From P_{II} the evolutionary trend branched, giving rise to *texana* on one hand by the 2–3 fusion and 5a inversion in addition, and to *novamexicana* on the other hand by Xc, 2bc, 3a, 4a and 5b, with no fusions of chromosomes. It has been suggested (Patterson 1941, Patterson, Stone and Griffen 1942) that *americana* arose by hybridization between *texana* and *novamexicana*, a suggestion we

shall discuss more fully later. Meanwhile we will accept this view for our discussion of inversion origins. *Drosophila americana* received the 2–3 fusion from *texana* and the Xabc from *novamexicana*. Apparently both the *texana* 4 and the *novamexicana* 4 have been preserved and spread in different directions, and onto the *novamexicana* 4a, *americana* has added 4b for its own, because 4b has never been observed independent of 4a. Xd and 4c are likewise of *americana* origin, and never have been transferred into any other species.

From P_I there also came a drastic change, i.e., the pericentric inversion, 2e. The fact that *littoralis* and all the members of the montana complex have 2ade and 4 def suggests another parental form P_{III} which later bifurcated into two branches, one giving rise to the montana complex in the new world and the other to *littoralis* in the old world by further changes. Therefore, phylogenetically *littoralis* is closer to the montana complex than it is to the americana complex. *Drosophila littoralis* decended from P_{III} that acquired a fusion of 3 and 4 and additional inversions on all the principal chromosome elements. We have very limited knowledge about *littoralis*, since we do not have enough samples of this European species. Perhaps there is also a number of species forming a littoralis complex in the old world. We know rather more about the diversification of the montana complex. Thus from P_{III} the parental form underwent a number of changes, all by inversions. The most complex chromosome is the X, which has so many inversions that we are not able to trace them in the hybrids of the montana complex with any of the species with less complicated X. The second, third and fifth have received 2fghjk, 3bc and 5cd respectively which made the basis for all the forms of the montana complex. The fourth chromosome is more complicated. Some of the *montana* strains retain the primitive P_{III} alignment (4def) with no more changes; others contain 4h. Since all the yampa and superior strains contain 4h, it is clear that they arose at the stage where some of the *montana* had gotten the 4h inversion. Further inversions in *montana*, viz., 3d, 4i, 4g and 4o did not enter into them, indicating a later appearance in the *montana* history. Onto the basic *montana* pattern (XM, 2adefghjk, 3bc, 4defh, 5cd and 6) yampa and superior added some specific inversions of their own and became isolated from *montana* to some extent. The situation of *lacicola* is slightly more complicated. Since 4h may or may not be present in the *lacicola* strains, it is questionable which original type of *montana* was the ancestor of *lacicola*. Either the form with 4def gave rise to *lacicola* and 4h got into *lacicola* by later hybridization, or vice-versa. Figures 7 to 12 are summaries of the inversions found in the virilis group species and Figure 12 is a diagrammatic summary in which most of the important changes are indicated. We put a "reversible reaction" sign for P_I and *virilis* to note the uncertainty of their phylogenetic sequence.

If the above discussion on the species relationships is correct, speculations can be put forward for the possible origin and diversifications of the species during the past. We have suggested that *virilis* was transported into the new world from the old continent. In China and Japan, or probably

Explanation of Figures 7–11

The inversions found on the five chromosomal elements are plotted with their positions on the chromosomes in proportion to the virilis salivary gland chromosome map. Species having the arrangements consistently are indicated at the right side of the chromosome with their initials in straight-type letters, whereas those may or may not possess the arrangements are in slant-type letters. A similar way is used for the inversions. Each inversion is in capital letter when it was originated, and in small letter when transferred into other forms or derivatives.

Symbols for Figures 7–12

V	*D.virilis*	S	D.*montana* superior strain
N	*D.novamexicana*	Lc	*D.lacicola*
A	*D.americana americana*	Li	*D.littoralis*
T	*D.americana texana*	P_I	Primitive form I
M	*D.montana*	P_{II}	Primitive form II
Y	*D.montana* yampa strain	P_{III}	Primitive form III

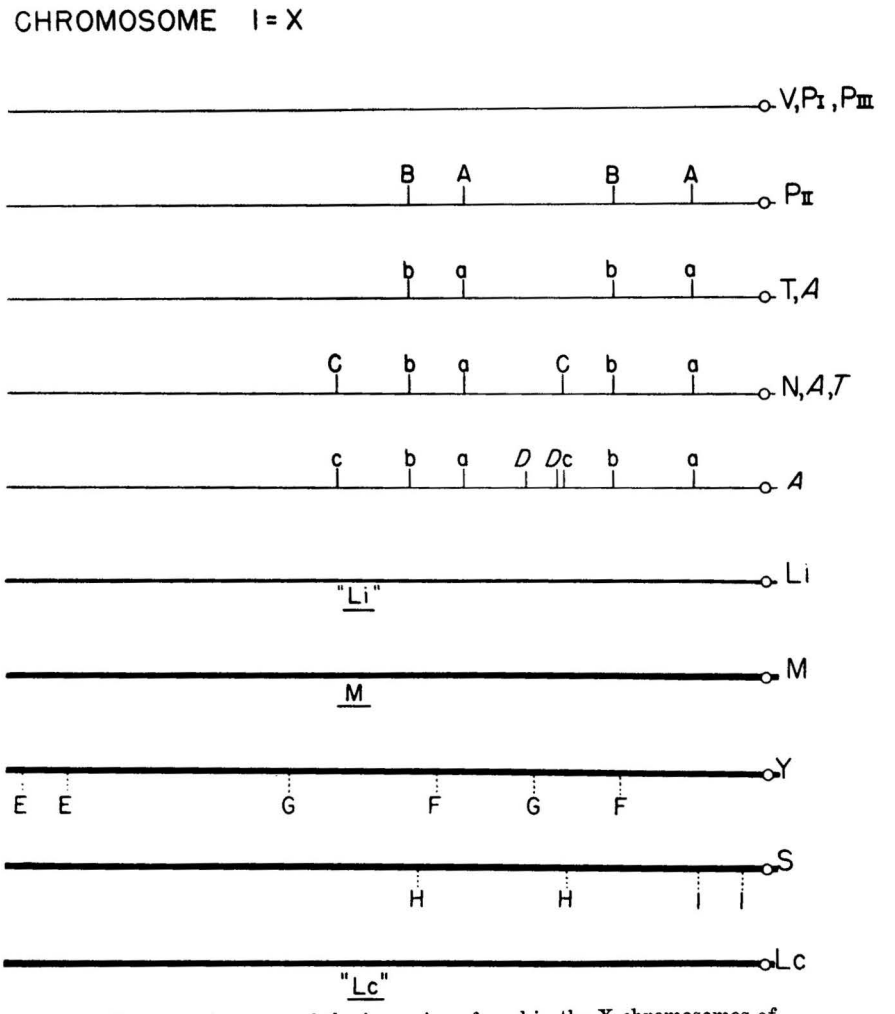


FIG. 7. Diagrams of the inversions found in the X-chromosomes of the virilis species group.

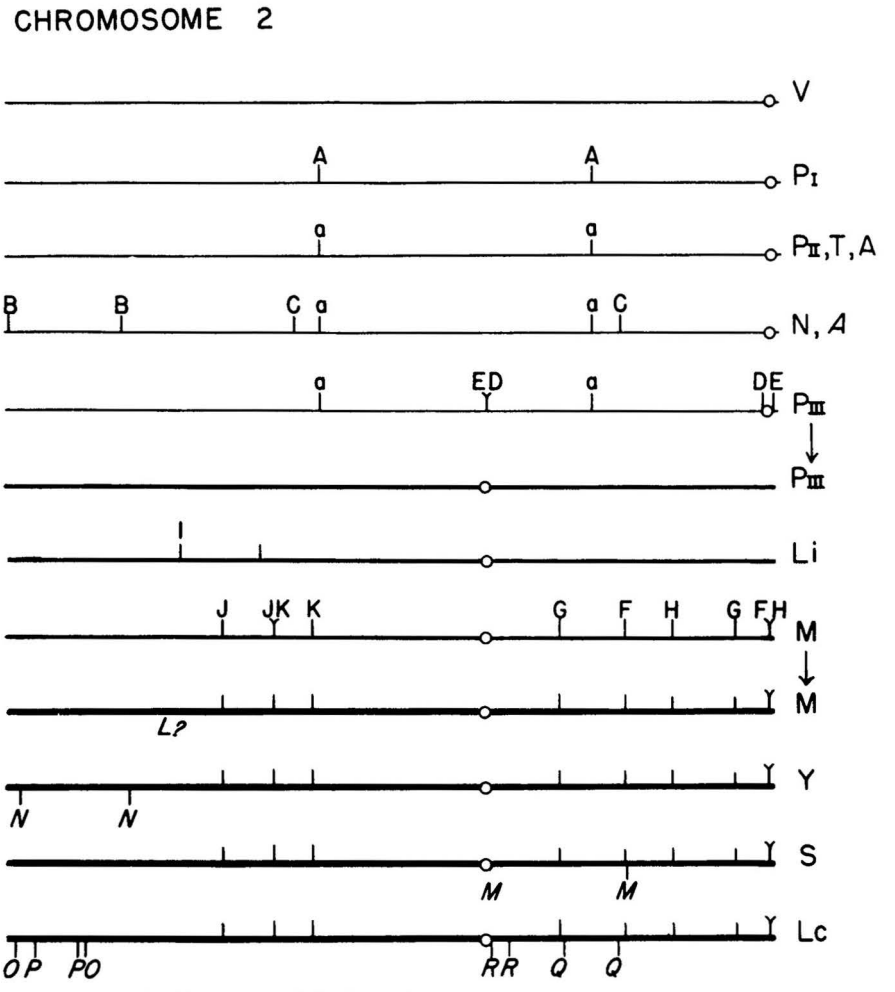


FIG. 8. Diagrams of the inversions found in the second chromosome of the virilis species group.

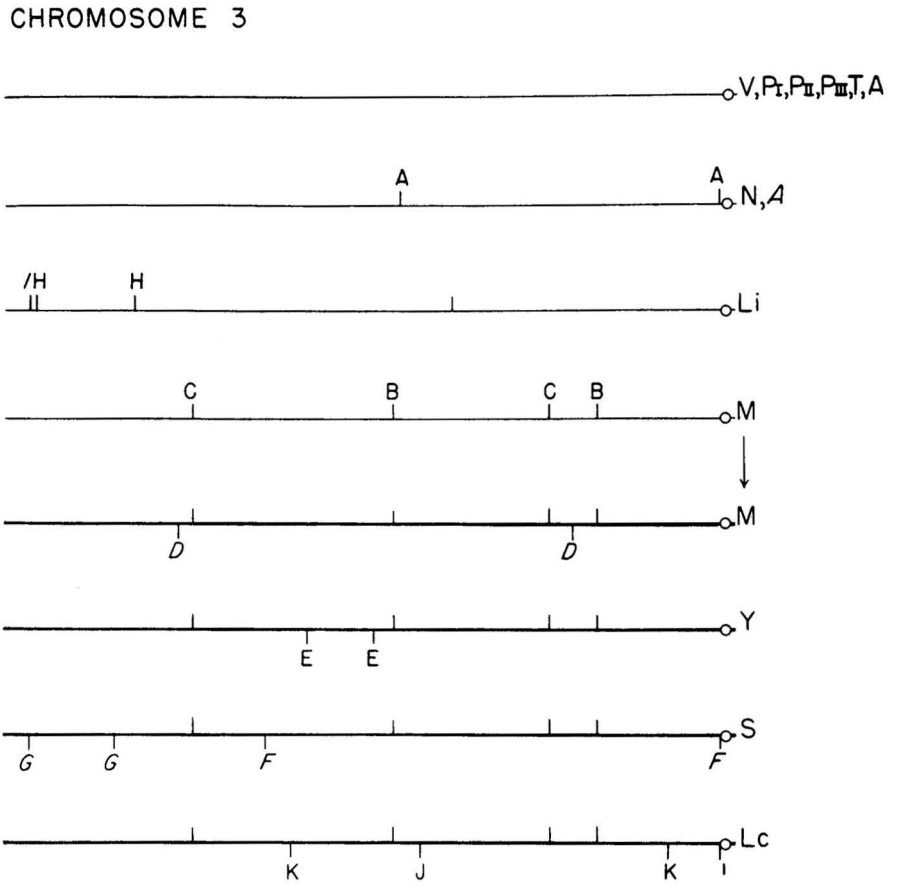


FIG. 9. Diagrams of the inversions found in the third chromosome of the virilis species group.

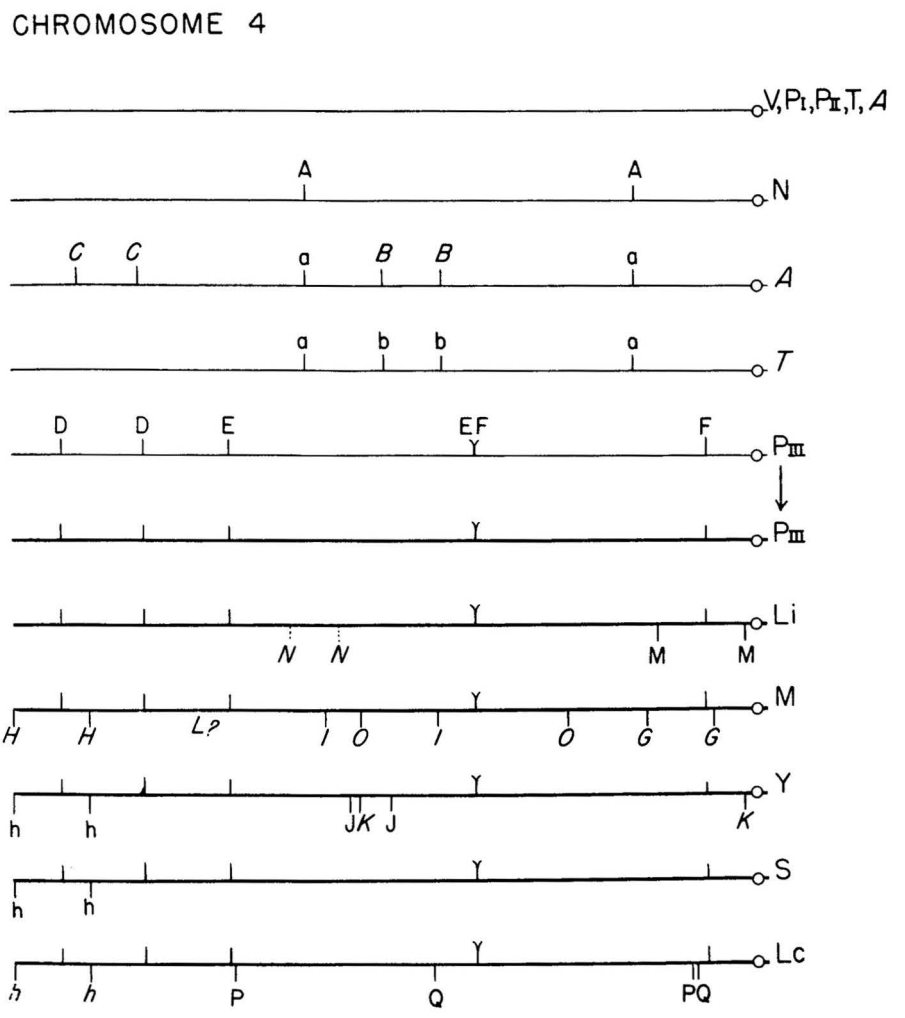


FIG. 10. Diagrams of the inversions found in the fourth chromosome of the virilis species group.

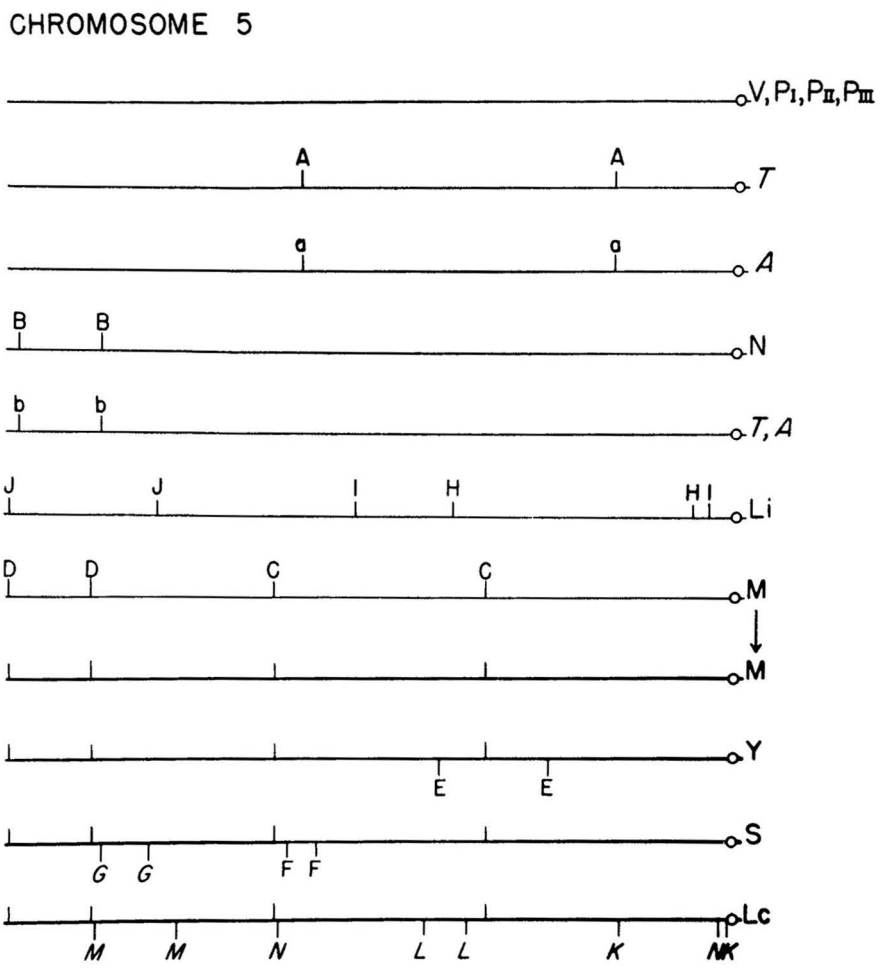


FIG. 11. Diagrams of the inversions found in the fifth chromosome of the virilis species group.

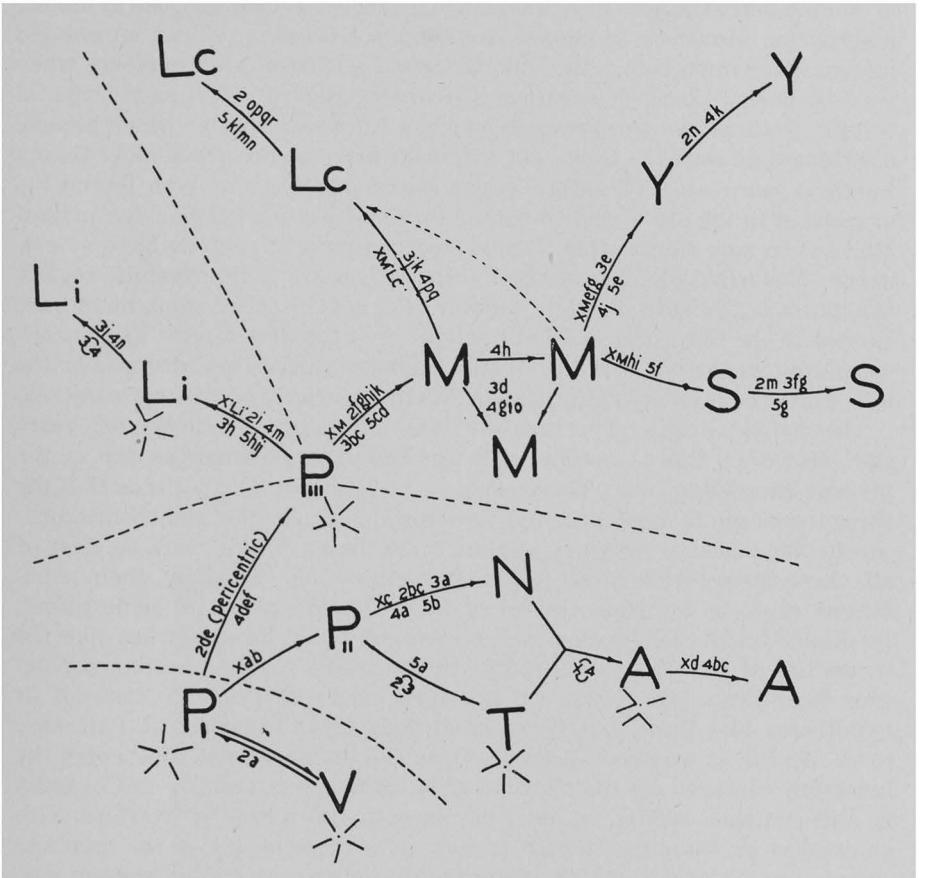


FIG. 12. Diagram of the cytological interpretation on the phylogeny of the virilis species group. For further explanations see text.

other parts of Asia, *virilis* is very common not only in the towns and cities, but in rural areas as well. However, it is extremely hard to differentiate wild and domestic conditions in China as we can do in the United States, because all over the fields and woods there are scattered houses and traces of human activities and one can always interpret a place as close to human association. However, in a place like Meitan, Kweichow, China, an isolated mountainous town before the war, we found *virilis* in large numbers when we first moved there. It is rather plausible to regard *virilis* as of Oriental origin. *Drosophila littoralis* is obviously a European species which became distributed all over the European continent and was described more than a hundred years ago. Therefore it also seems possible that both P_I and P_{III} originated in the old world. Whether P_{II} was from the old world or originated in the new world after P_I migrated to this continent we have no evidence. Nevertheless, since all the P_{II} derivatives are in the Nearctic region, the latter is probably the right answer. P_{III}, on the other hand, must have existed in the old world and later migrated to the new world. The branch remaining in the old world gradually metamorphized into *littoralis* or the like while the immigrants settled in North America and became *montana.*

The hybrid origin of *americana* was suggested a number of years ago. However, this is not the only possible interpretation as far as the present knowledge goes. There could be an alternative hypothesis that the three forms are in a balanced population which shifts now and then according to the selection pressure applied upon them. If this were so, first of all, these three forms would not be at species rank. Secondly, their populations could be mingled, i.e., all of them should overlap in some places. In other words, the fusions and inversions would be somewhat like the inversions of *pseudoobscura* which are the means for maintaining population flexibilities. Of course we can seek no direct proof or disproof of hypotheses like these, but the present data favor the view of Patterson *et al.* As far as we know, *novamexicana* is a stable species concerning the inversion content. Its distribution area, although extending to Colorado, is still limited; and no locality has been found where it overlaps with *americana* or *texana,* although it does so with members of the montana complex in Colorado, which is apparently irrelevant to our present discussion. In other words, *novamexicana* is in a highly isolated situation to its relatives and perhaps it has been so for a long while.

Genetic tests made by Patterson and Stone (1949) show that *novamexicana* is sexually highly isolated also, and it is regarded as a good species. If the whole population of the americana complex is still in a balanced condition, some of the *texana* rearrangements, e.g., 5a, or *americana* rearrangements like Xd, 4b, 4c, might be transferred into *novamexicana.* The fact they did not would mean that *novamexicana* has been isolated much earlier than the time at which Xd, 4b and 4c originated.

The discovery of the western *americana* and the presence of some of the *novamexicana* inversions in these populations lead us to make some supplementary suggestions to Patterson *et al.'s* hypothesis. If we accept their view we have to admit that after the hybridization of *novamexicana* and

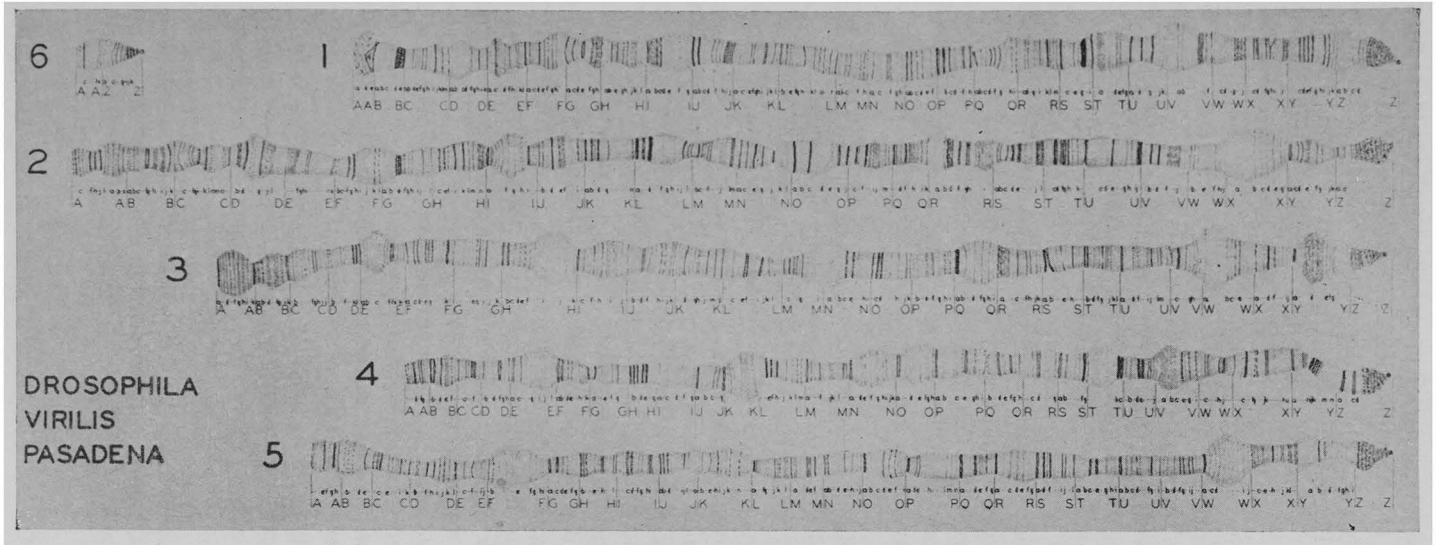


FIG. 13. Chromosome map of *Drosophila virilis*.

texana, there was a long period of over-lap of all the three forms. It is more likely that after the hybridization the new hybrid *americana* spread north along the Mississippi Valley. Its eastern branch extended along the Ohio River, through Illinois, Indiana, Ohio, Pennsylvania and up to Michigan, New York and Vermont. The flies repeatedly hybridized with *texana* along the border zone and exchanged their genic and chromosomal contents with *texana*. The western branch, on the other hand, may have spread along the Missouri River, from Kansas via Nebraska, the Dakotas up to Montana. Along the western border of this region (presumably the Platte and the Arkansas River Valleys) they might have met their other ancestral species, *novamexicana*, and likewise hybridized with it. Therefore the chromosomal contents of the eastern *americanas* are more like those of *texana*, and that of the western *americanas*, more like *novamexicana*. But the time of overlapping of *americana* and *novamexicana* might have come to an end much sooner, and the *novamexicana* inversion 2c and 3a might not be selectively favorable for *americana* (especially as *americana* has the *texana* 2–3 fusion, which might reduce the crossing over with the unfused rods). These inversions are thus in an extremely low frequency as compared to others.

On the other hand, eastern *americana* and *texana* still exchange genes at the present time to a limited degree. Sequences such as 4a, 4ab, 5b, etc., were probably transferred to the eastern *americana* from the western branch, and then transferred to *texana*. Therefore in *texana* only those strains in the overlapping zone or close to it can have Xc, 4ab, or 5b, whereas the strains from the deep south have typical *texana* arrangements.

Since P_{III} originated in the Palaearctic region, it must have migrated over to the Nearctic region while the two mainlands were still united. The fact that the montana strains from the western coast of the United States, e.g., California and Nevada, still retain the primitive 3 and 4 arrangements suggests the route taken by P_{III} was probably through British Columbia and down to the coastal states. When the species *montana* was established, they migrated eastwards again and found the Rocky Mountain region in which to settle down. There they evolved more changes, spread rapidly and gave rise to the montana complex. We have no idea how *montana* diversed into other species in the complex. Very possibly all the forms arose from *montana* in the Rocky Mountain region and spread elsewhere thereafter. The sympatric habitat of yampa and superior and *lacicola* and superior is apparently a later development, after the isolation between the forms had been established.

SUMMARY

These cytological studies are in agreement with conclusions of Patterson and Stone, that the nine known forms in the virilis species group should be classified as follows:

1) *D. virilis*
2) The americana complex (three forms)

3) *D. littoralis*
4) The montana complex (four forms)

Using the salivary chromosome map of *virilis* as a standard, all the other forms show a considerable number of inversions in all the five principal chromosome elements, especially the X, which has undergone changes so profound that we are not able to trace the sequences in some species.

All the traceable inversions are symbolized, briefly described, and their approximate breakage points determined.

With the exception of *virilis* and *novamexicana* all the other species have variations in their gene arrangements among stocks.

Different species may have common inversions. This fact enables one to interpret the phylogenetic relationships of the group. The major scheme of the phylogeny has been suggested by the inversion tracers. There was a hypothetical primitive form P_I (containing inversion 2a against *virilis*) which gave rise to P_{II} by inversions Xab, and to P_{III} by 2de (one of them is pericentric) and 4def.

P_{II} was the parental form of the americana complex. *D. texana* arose by a fusion between 2 and 3 plus inversion 5a, and other inversions with no fusion gave rise to *novamexicana*. The present data do not contradict the hybrid origin hypothesis of *americana* first proposed by Patterson (1941) and later expanded by Patterson, Stone, and Griffen (1942).

P_{III} was the ancestral form of both *littoralis* and the montana complex. From P_{III}, a fusion between 3 and 4 and other inversions occurred in *littoralis;* and in another line many other inversions (no fusions) gave rise to *montana*. Some strains of *D. montana* still represent the most primitive type of all the montana complex, but others are more highly evolved. From *montana* with 4refh, yampa and superior branched out and evolved their own variations. *D. lacicola* probably came from the most primitive *montana* ancestor, but the sequence 4h also entered into *lacicola*, presumably by hybridization while the two species were not well isolated. Possible origins and their trend of evolution of these forms as well as the primitives are discussed.

BIBLIOGRAPHY

Burla, H. 1951. Systematik, Verbreitung und Oekologie der Drosophila-Arten der Schweiz. Revue Suisse de Zoologie, 58:23–176.

Chino, M., and H. Kikkawa. 1933. Cytological demonstration of crossing over in the autosomes of *Drosophila virilis*. Cytologia, 4:453–456.

Dobzhansky, Th. 1941. Genetics and Origin of Species. Columbia U. Press.

Dobzhansky, Th. 1948. Genetics of natural populations. XVI. Altitudinal and seasonal changes produced by natural selection in certain populations of *Drosophila pseudoobscura* and *Drosophila persimilis*. Genetics, 33:158–176.

Dobzhansky, Th., and C. C. Tan. 1936. Studies on hybrid sterility. III. A comparison of the gene arrangement in two species, *Drosophila pseudoobscura* and *Drosophila miranda*. Zeit. f. Ind. Abst. Vererb., 72:88–114.

Fujii, S. 1936. Salivary gland chromosomes of *Drosophila virilis*. Cytologia, 7:272–275.

Fujii, S. 1942. Further studies on the salivary gland chromosomes of *Drosophila virilis*. Cytologia, 12:435–459.

Horton, I. H. 1939. A comparison of the salivary-gland chromosomes of *Drosophila melanogaster* and *D. simulans*. Genetics, 24:234–243.

Hsu, T. C. 1950. Cytological studies in the virilis group of Drosophila. I. Inversion variations in the *americana-texana-novamexicana complex*. Genetics, 35:114–115.

Hughes, R. D. 1936. The morphology of the normal salivary chromosomes of *Drosophila virilis*. J. Hered., 27:305–306.

Hughes, R. D. 1939a. The chromosomes in the hybrid between *Drosophila virilis virilis* and *Drosophila virilis americana* Spencer. Genetics, 24:99.

Hughes, R. D. 1939b. An anlaysis of the chromosomes of two subspecies, *Drosophila virilis virilis* and *Drosophila virilis americana*. Genetics, 24:811–834.

Mayr, E. 1942. Systematics and Origin of Species. Columbia U. Press.

Patterson, J. T. 1941. The virilis group of Drosophila in Texas. Amer. Nat., 75: 535–539.

Patterson, J. T. 1942a. Interspecific hybridization in the genus Drosophila. Univ. Texas Publ., 4228:7–15.

Patterson, J. T. 1942b. Distribution of the virilis group in United States. Univ. Texas Publ., 4228:153–161.

Patterson, J. T. 1944. A new member of the virilis group. Univ. Texas Publ., 4445:102–103.

Patterson, J. T. and A. B. Griffen. 1944. Relationships of *Drosophila montana* and *D. lacicola* to other members of the virilis group. Univ. Texas Publ., 4445:194–211.

Patterson, J. T. and W. S. Stone. 1949. The relationship of *novamexicana* to the other members of the virilis group. Univ. Texas Publ., 4920:7–17.

Patterson, J. T. and W. S. Stone. 1952. Evolution in the genus Drosophila. Macmillan, New York (in press).

Patterson, J. T., W. S. Stone and A. B. Griffen. 1940. Evolution of the virilis group in Drosophila. Univ. Texas Publ., 4032:218–241.

Patterson, J. T., W. S. Stone and A. B. Griffen. 1942. Genetic and cytological analysis of the virilis species group. Univ. Texas Publ., 4228:162–183.

Patterson, J. T. and R. P. Wagner. 1943. Geographical distribution of species of the genus Drosophila in the United States and Mexico. Univ. Texas Publ., 4313:217–281.

Patterson, J. T. and M. R. Wheeler. 1942. Description of new species in the subgenera Hirtodrosophila and Drosophila. Univ. Texas Publ., 4213:69–109.

Simpson, G. G. 1944. Tempo and Mode in Evolution, Columbia U. Press.

Sokolov, N. N. 1948. A new species of Drosophila—*Drosophila imeretensis*. Comp. Rend. (Dokl.) Acad. Sci. U.S.S.R., 59, No. 5:1007–1008.

Spencer, W. P. 1938. *Drosophila virilis americana*, a new subspecies. Genetics, 23:169–170.

Stalker, H. D. 1940. Chromosome homologies in two subspecies of *Drosophila virilis*. Proc. Nat. Acad. Sci., 26:575–578.

Stone, W. S. and J. T. Patterson. 1947. The species relationships in the virilis group. Univ. Texas Publ., 4720:157–160.

Sturtevant, A. H. 1916. Notes on North American Drosophilidae with descriptions of twenty-three species. Ann. Ent. Soc. Amer. 9:323–343.

Tan, C. C. 1935. Salivary gland chromosomes in the two races of *Drosophila pseudoobscura*. Genetics, 20:392–403.

Warters, M. 1944. Chromosomal aberrations in wild populations of Drosophila. Univ. Texas Publ., 4445:129–174.

Wharton, L. T. 1942. Analysis of the repleta group of Drosophila. Univ. Texas Publ., 4228:23–52.

Wharton, L. T. 1943. Analysis of the metaphase and salivary chromosome morphology within the genus Drosophila. Univ. Texas Publ., 4313:282–327.

White, M. J. D. 1945. Animal Cytology and Evolution. Cambridge U. Press.

APPENDIX

List of Inversions

For each inversion the approximate breakages on the *virilis* map are indicated. The species which have the sequence consistently are indicated with their initials, those

which may not have the sequence are in parentheses. Independent inversions have their breakage points according to the *virilis* map order, *e.g.*, inversion 4f has the distal break between 4Qf and 4Qg (4Qf-g) and the proximal break between 4Yd and 4Ye (4Yd-e). If one inversion is superimposed on another, the breakage points are indicated in reverse order. For example, inversion 4g is overlapping 4f, its distal break is thus reversed, being 4Xc-b instead of 4Xb-c; but its proximal break is not influenced by any previous inversions, it is still in the original order. Included inversions may have both the break points reversed.

The X-chromosome

Symbol	Found in	Descriptions	Approximate Breakages Distal	Proximal
a	TAN	submedian	Qb-c	Ye-f
b	TAN	overlaps a	Nf-g	Vc-b
c	N(T)(A)	overlaps a, b	Lg-h	Te-f
d	(A)	included in c	Td-c	Se-d
e	Y	median on XM	unknown	unknown
f	Y	overlaps e	unknown	unknown
g	Y	subterminal on XM	unknown	unknown
h	S	median on XM	unknown	unknown
i	S	basal on XM	unknown	unknown
"Li"	Li	X of *littoralis*	many inversions	
M	M	X of *montana*	many inversions	
M"Lc"	Lc	X of *lacicola*	many inversions on XM	

Chromosome 2

a	all except V.	median	Ki-j	T*l*-Ua
b	N(A)	subterminal	Ac-d	Dh-i
c	N(A)	includes a	Jf-g	Ug-h
d	MYSLcLi	basal, overlaps a	Pn-o	Z
e	MYSLcLi	pericentric, overlaps ad	near Po	other side of centromere
f	MYSLc	superimposed on ade	term.het.2R	Ub-c
g	MYSLc	superimposed on adef	Le-d	?
h	MYSLc	superimposed on adefg	Wa-Vj	near Ub-c
i	Li	distal 2L	Fj-k	Ig-h
j	MYSLc	distal 2L	He-f	near I-J
k	MYSLc	distal 2L	near I-J	Kg-h
l	(M)	distal 2L	unknown	unknown
m	(S)	base to subt. 2R	Uh-i	Z
n	(Y)	subterminal 2L	Ai-j	D*l*-m
o	(Lc)	subterminal 2L	Ac-d	Cj-k
p	(Lc)	included in o	Ci-h	Be-d
q	(Lc)	subterminal 2R	term.het.	V
r	(Lc)	basal 2R	?	Z

Chromosome 3

a	N(A)	basal	Ng-h	Z
b	MYSLc	median	Nb-c	Vd-e
c	MYSLc	overlaps b	Hc-d	Tc-b
d	(M)	overlaps c	Gi-j	Ub-c
e	Y	included in c	Mh-g	Kj-i
f	(S)	basal, overlaps bc	Jd-e	Z
g	(S)	subterminal	Bc-b	Ej-k
h	Li	subterminal	Bc-d	Fe-f
i	(Li)	includes h	Ak-Ba	Pf-g
j	Lc	basal, overlaps bc	Oe-d	Z
k	Lc	overlaps j	Kc-b	Ya-b

Chromosome 4

a	N(A)(T)	median	Fg-h	Wh-i
b	(A)(T)	included in a	Pc-b	Nf-e
c	(A)	distal	Db-c	Fe-f
d	MYSLcLi	subterminal	Cf-g	Fh-i

Symbol	Found in	Descriptions	Approximate Breakages Distal	Proximal
e	MYSLcLi	median	Ib-c	Qf-g
f	MYSLcLi	submedian	near Qf-g	Yd-e
g	(M)	subbasal, overlaps f	Xc-b	Y*l*-m
h	YS(M)(Lc)	terminal, overlaps d	Ab-c	Dh-g
i	(M)	included in e	Pb-a	Ma-Lm
j	Y	included in e	Oe-d	M*l*-k
k	(Y)	basal, overlaps efj	Na-b	Z
l	(M)	distal	unknown	unknown
m	Li	subbasal, overlaps f	Xh-g	Yn-Za
n	(Li)	included in e	Ng-f	Le-d
o	(M)	overlaps e, f	Mm-*l*	T
p	Lc	overlaps e, f	Km-*l*	Id-c
q	Lc	overlaps p	Ks-t	Pa-b
		Chromosome 5		
a	(T)(A)	median	Kb-c	Vf-g
b	N(T)(A)	subterminal	Af-g	Dk-*l*
c	MYSLc	submedian	Ik-Ja	Rb-c
d	MYSLc	subterminal	Ac-d	Dj-k
e	Y	overlaps c	Pf-e	Tf-g
f	S	included in c	Kn-*l*	Jj-i
g	(S)	distal	D*l*-Ea	Ff-g
h	Li	large basal	Lk-*l*	Z
i	Li	subbasal, included in h	Yd-c	P*l*-k
j	Li	subterminal	Ac-d	Fg-h
k	(Lc)	basal	Wa-b	Z
l	(Lc)	included in c	Qc-b	Og-f
m	(Lc)	distal, independent	Eg-i	H*l*-m
n	(Lc)	subbasal, overlaps c	?	?

IV. GENE VARIABILITY IN THE AMERICANA-TEXANA-NOVA-MEXICANA COMPLEX OF THE VIRILIS GROUP OF DROSOPHILA

MARY L. ALEXANDER

INTRODUCTION

Mutation studies are necessary for a more complete understanding of the process of evolution. The only proven, constant method for a continuation of variation is that of mutation. Such a basic material of evolution is not, however, independent of such factors as isolation, selection and environmental conditions. These are interacting, interdependent factors and any one or combination of these can vary in importance in different situations.

The study of evolution by use of Drosophila, as any other living form, demands a consideration of the characteristics of the genus and even the species used. Distribution, physiological peculiarities, the length of the life cycle, food supply and preference, fluctuation of population size and resistance to environmental changes cannot be disregarded.

The importance of mutations and geographical variation in evolution has been reviewed by Timofeeff-Ressovsky (1940). General consideration of gene mutation in Drosophila can be found in a review by Spencer (1947a). Studies of the evolution within the genus Drosophila and especially in the virilis group have been published in the University of Texas Publications (see references to Patterson and Stone).

The present investigation is designed to measure that gene variability which lies within the range of morphological detection found in natural populations of three members of the virilis group of Drosophila. Two of these, *Drosophila americana americana* Spencer (1938) and *Drosophila a. texana* Patterson, Stone and Griffen (1940), are closely related genetically since natural hybrids have been found in the overlap zone of the distribution ranges (Stone and Patterson, 1947). These two members of the virilis complex have reached a level of divergence of subspecies but the third member of this division, *Drosophila novamexicana* Patterson (1941), has been designated as a species (Patterson and Stone, 1949). For convenience of discussion "species" will be used to refer to any one of these three members of the group.

The use of these three closely related species allows a comparative study of the gene variability and general mutation structure of the natural populations of each. The populations of *americana* and *texana* are usually termed "medium-sized" thus being smaller than those of such species as *D. melanogaster* or *D. hydei* but larger than *D. limpiensis* populations. The *novamexicana* populations are much smaller than those of *americana* or *texana*.

Studies of morphological variation which exist within natural populations of Drosophila are rather incomplete and fragmentary at the present

time. This type of study measures the variability present in the form of relatively minor changes.

MATERIAL AND METHODS

The stocks tested for this investigation were obtained by field collections of natural populations. The three forms used, *americana, texana* and *novamexicana,* occur as "wild" populations as opposed to the "domestic" *virilis.* All of the laboratory and wild strains used are listed in Table 1.

TABLE 1

Laboratory and wild strains of *americana, texana* and *novamexicana* used in this study

Species	Stock number	Date of collection	Place of collection	Remarks
americana	2069.7	8-22-50	Hastings, Nebraska	wild strain
americana	1773.4e	8-8-47	Chadron, Nebraska	lab. strain
americana	2067.1	8-20-50	Chadron, Nebraska	wild strain
americana	2068.6	8-21-50	Oakdale, Nebraska	wild strain
americana	1760.8f	7-25-47	Poplar, Montana	lab. strain
texana	1128.10	6-15-41	New Orleans, La.	lethal free
texana	2007.6	6-12-50	Tallahassee, Fla.	wild strain
texana	2012.4	6-19-50	Keystone Heights, Fla.	wild strain
texana	2013.3	6-20-50	Lake Butler, Fla.	wild strain
texana	2014.1'	6-20-50	Twin Lakes, Georgia	wild strain
texana	2015.4	6-21-50	Indian Springs, Ga.	wild strain
texana	2016.7	6-22-50	Acworth, Georgia	wild strain
texana	2017.4	6-23-50	Smokemont Camp, N. C.	wild strain
texana	2018.7	6-25-50	Trenton, Georgia	wild strain
texana	2019.1	6-25-50	Guntersville, Ala.	wild strain
texana	2020.1	6-26-50	Tupelo, Mississippi	wild strain
texana	2021.6	6-27-50	Hollandale, Miss.	wild strain
novamexicana	1714.4	6-16-47	San Antonio, N. M.	lethal free
novamexicana	2075.8	8-29-50	Cliff, New Mexico	wild strain

The procedure of testing individual flies from natural populations for morphological mutants necessarily varied somewhat with the different populations of the three species of Drosophila tested. Since a majority of the mutations carried in such populations are recessive, the general procedure consisted in obtaining F_1 offspring from the P_1 crosses and inbreeding the F_1's in pairs or in mass matings depending upon the number available for such test. The F_1, F_2 and subsequent generations were checked for morphological mutations.

For paired matings of F_1 individuals, virgin females were used; mass matings usually consisted of from ten to twenty pairs of virgin or non virgin flies. Most of the larvae produced from P_1 and F_1 matings were spread to fresh food to eliminate overcrowding of the vials and thus prevent selection against homozygous mutants if these were less viable than normal.

Three methods of obtaining F_1 offspring were used. The test males and females of the same population were inbred in pairs or outcrossed individually to a standard laboratory strain. In some cases offspring were collected from females which had been fertilized in nature previous to collection. The latter were designated as "iso" females.

Each test male and female was arbitrarily given a different letter, as a, b, c, etc., at the beginning of the experiment. The number and type of P_1 individuals tested from each population of the three species has been included in Tables 2, 3, 4, and 5. The number of F_1 paired matings which were checked for each tested individual or individuals has also been indicated.

RESULTS

The morphological mutations detected in *americana, texana* and *novamexicana* have been assigned descriptive names and are arranged in alphabetical order. This list, which also contains a brief description of the more interesting mutants, has been placed in the Appendix. Mutants which were not tested and were not particularly important in the present analysis are sometimes included under a general name as *rough.* The allelic mutants are designated as a, b, c, etc., in the distribution tables. When numbers as 1, 2, 3, etc., follow the mutant name, no allele tests were possible or have not been completed. Specific names as *roughoid* were assigned to those mutants which have been tested to other morphological similar mutants of the same or different populations.

The names which were used for mutants were based on the terminology used for the mutants of the better known species of this group, *D. virilis* Sturtevant. Since crosses between *virilis* and *americana, texana,* or *novamexicana* are possible, the linkage group and allelism of some mutants of these species have been established. When it was not possible to check allelism to *virilis* descriptive names based upon morphological similarity to mutants of this species were used. If the recovered mutants did not resemble any known *virilis* mutants, the names of phenotypically similar melanogaster mutants were used.

The distribution of mutants in populations of *americana, novamexicana* and *texana* are found in Tables 2, 3, 4 and 5. Table 2 is composed of three *americana* populations and one *novamexicana* population. The more extensive data from *texana* populations are recorded in Tables 3, 4 and 5. Females which were fertilized in nature previous to collection are designated as "iso" females in the tables and are treated in the final analysis as a mating of one male and one female of the same population. Although multiple fertilization by several males can occur, it is justifiable to assume only one male since the rate of replacement of the sperm from pervious inseminations by the sperm of a male which fertilized the female last is very rapid and very complete. The efficiency and rate of sperm replacement in crosses between *texana, americana* and *virilis* have been reported by Patterson, Stone and Griffen (1940). Additional tests for the rate of replacement in *americana* and *texana* were carried out by use of the *cinnabar* mutants. The *cinnabar* females from pure mutant stocks were isolated individually in vials. After larvae appeared in the first vial, the mutant female was crossed to a normal male of the standard strain. A *cinnabar* female and normal male were left in the second vial for four days and then transferred to a third vial. In all cases the first vial which was a control

TABLE 2: MUTANT DISTRIBUTION IN POPULATIONS OF DROSOPHILA AMERICANA AMERICANA AND DROSOPHILA NOVAMEXICANA

Stock Number	P1's Tested	Number of F1 Pairs Checked	Mutants Recovered: Eye Color	Body Color	Wing Size	Wing Veins	Bristle Mutants	Rough Eyes	Other Body Structures	Multiple Effects
AMERICANA 2067.1	a (iso ♀)	11		Light-1			Extreme-like: Hairless		Everted-1	Rough-cut
	b (iso ♀)	12		Light-2	Tapering	Interrupted-1		Varnished		Slight-1
	c (iso ♀)	2		Light-3			Double			
	e (iso ♀)	8		Light-4		Interrupted-2				
	h (iso ♀)	7		Light-5		Interrupted-3			Everted-2	
	i (iso ♀)	Mass		Light-6						
	k (iso ♀)	11	Red	Light-7		Interrupted-4				
	m (iso ♀)	2	Cinnabar	Light-8					Downcast-sterile	
AMERICANA 2068.6	b (iso ♀)	16				Interrupted-5	Extreme-1: Small bristles-1: Strand-1			Displaced
	d (iso ♀)	7		Light-9	Constricted like-1	Interrupted-6			Spread-1	Slight-2
	e (iso ♀)	4			Constricted like-2: Arched		Small bristles-2			
	f (♀ x Sd*)	Mass			Wide					
	g (♀ x Sd*)	Mass		Light-10						
	h (iso ♀)	8	Orange			Interrupted-7	Strand-2		Tinted	
	l (♀ x Sd*)	7		Light-11						Weak Mosaic-1
	o (iso ♀)	7	Wine-1	Yellow	Parallel					
	p (iso ♀)	5	Wine-2			Interrupted-8	Blunt		Grooveless-like: Swollen	
	j♀ x a♂	13		Light-12	Narrow					Abruptex: Mosaic-2
AMERICANA 2069.7	a (iso ♀)	3		Light-13		Interrupted-9				
	b (iso ♀)	Mass							Increased	
	c (iso ♀)	Mass							Immature: Spread-2	
	f (iso ♀)	Mass							Aristapedia-like	
	g (iso ♀)	8	Wine-3	Light-14				Rough	Everted-3	
	n (iso ♀)	4		Light-15					Swollen	
	o (iso ♀)	6		Light-16					Bubble	Rough-spread
	d♀ x d♂	9		Light-17						
	i♀ x e♂	9		Light-18	Constricted	Abrupt	Extreme-2			
	j♀ x b♂	12			Pointed			Roughex		
NOVAMEXICANA 2075.8	a♀ x a♂	12				Broken-1				
	b(♀ x Sd**)	8				Broken-2			Curved	
	c(♀ x Sd**)	9								Shaggy
	d(♀ x Sd**)	14								
	e (iso ♀)	16								
	f (iso ♀)	7					Sparse			
	g(♀ x Sd**)	12								
	h(♀ x Sd**)	19			Shortened					

* Laboratory Strain of D. americana, 1760.8f
** Laboratory Strain of D. novamexicana, 1714.4

TABLE 3: MUTANT DISTRIBUTION IN DROSOPHILA AMERICANA TEXANA: INDIAN SPRINGS, GEORGIA

		Mutants Recovered								
F1's Tested	Number of F1 Pairs Checked	Eye Color	Body Color	Wing Size	Wing Veins	Bristle Mutants	Rough Eyes	Wing Structure	Other Body Structures	Multiple Effects
1a (iso 9)	3					Absent-5	Rougher			
1c (iso 9)	7									
1g (iso 9)	4									Rough-cut
1h (iso 9)	Mass									
1k (iso 9)	2					Small bristles-1				
1a (♂ x S9*)	1			Pointed-1		Small bristles-3	Rough-7			
1b (♂ x S9)	10			Pointed-2	Short 4th Gapped		Roughoid-2	Spread-2		
1c (♂ x S9)	13				Short veins-b	Small-absent-1				
1d (♂ x S9)	16									
1e (♂ x S9)	9									
1f (♂ x S9)	5							Ragged-7	Grooveless-1	
1g (♂ x S9)	2									
1h (♂ x S9)	15									Dishavelled: Small-extra: Rough-grooveless
1i (♂ x S9)	8									
1j (♂ x S9)	8				Short 5th-5		Roughened			
1k (♂ x S9)	4					Small bristles-4: Absent-6				Dumpy-2
1m (♂ x S9)	9									
1n (♂ x S9)	5									
1o (♂ x S9)	3							Ragged-5		Absent-semile thal
1p (♂ x S9)	5									
1q (♂ x S9)	12							Downcast-1		
1s (♂ x S9)	7			Pointed-3					Abnormal Abdomen-1	
1t (♂ x S9)	4									
1u (♂ x S9)	17								Abnormal Abdomen-2	
1w (♂ x S9)	8									
1x (♂ x S9)	6	Mahogany-3			Short veins-c	Small-absent-2		Ragged-6		
1y (♂ x S9)	8								Haltere	
1z (♂ x S9)	12					Scutullar-like: Hooked				
2a (♂ x S9)	11					Small bristles-5	Rough-8	Downcast-2		
2c (♂ x S9)	12							Downcast-3		
2e (♂ x S9)	7									
2h (♂ x S9)	7			Diminished-a			Rough-9			
2i (♂ x S9)	3				Short 5th-6					
2z (♂ x S9)	4			Small wing-1						
1e ♀ x 1b ♂	5			Pointed-like			Roughoid-1			
1k ♀ x 1k ♂	5			Narrow		Small bristles-6				Dumpy-2
1n ♀ x 1d ♂	14	Brilliant			Short veins-a	Small bristles-2		Extended-2		
1o ♀ x 1p ♂	3							Spread-1		

* Laboratory Strain of D. texana, 1128.10

TABLE 4: MUTANT DISTRIBUTION IN DROSOPHILA AMERICANA TEXANA

P_1's Tested	Number of F_1 Pairs Checked	Mutants Recovered								
		Eye Color	Body Color	Wing Size	Wing Veins	Bristle Mutants	Rough Eyes	Wing Structure	Other Body Structures	Multiple Effects
TALLAHASSEE, FLORIDA										
1a (♂ x S♀)*	6					Small bristles-a				
1b (♂ x S♀)	10				Short 5th-1		Roughest			
1c (♂ x S♀)	5	Mahogany-1					Rough-1			
1d (♂ x S♀)	4				Veinlet	Absent-1	Rough-2			Bithorax
1e (♂ x S♀)	4									Rough-extreme
1f (♂ x S♀)	5					Absent-2: extreme				
1g (♂ x S♀)	9				Short 5th-2		Rough-3	Ragged-1	Mottled	
1n (♂ x S♀)	9	Mahogany-2			Thickened	Missing-like: Small bristles-b		Extended-1		
1b♀ x 1b♂	6	Scarlet-like					Rough-4		Closed	
1j♀ x 1k♂	3									
TUPELO, MISSISSIPPI										
1a (iso ♀)	4	Bright-1								
1b (iso ♀)	3									Spread-semilethal
1f (iso ♀)	-				Plexus					
1g (iso ♀)	4					Small bristles-7			Abnormal Abdomen-3	
1a (♂ x S♀)	12								Stocky-1	Rough-mottled
1b (♂ x S♀)	10								Stubby-1	
1c (♂ x S♀)	4									
1d (♂ x S♀)	9			Pointed-4						
1f (♂ x S♀)	6		Ebony							
1g (♂ x S♀)	8				Short 5th-7	Small bristles-8	Rough-10			
1d♀ x 1f♂	9		Ebony	Fan	Abruptoid					Rough-broad
1h♀ x 1b♂	5								Stubby-2	Rough-short
1h♀ x 1d♂	6	Cinnabar-c				Irregular				
1e♀ x 1e♂	1	Bright-2								
1i♀ x 1a♂	7						Fused		Stocky-2	

* Laboratory Strain of D. texana, 1128.10

TABLE 5: MUTANT DISTRIBUTION IN DROSOPHILA AMERICANA TEXANA

Stock Number	P_1's Tested	Number of F_1 Pairs Checked	Mutants Recovered								
			Eye Color	Body Color	Wing Size	Wing Veins	Bristle Mutants	Rough Eyes	Wing Structure	Other Body Structures	Multiple Effects
Keystone Heights, Fla. 2012.4	a (♂ x S♀*)	5							Strap		Rough-absent
	b (♂ x S♀)	2					Small: Absent-3		Ragged-2		
	c (♂ x S♀)	4									Narrow-broken: Rough-vestigial
Lake Butler, Fla. 2013.3	a (♂ x S♀)	Mass			Wide			Rough-5	Blister		
Twin Lakes, Ga. 2014.1	a (♂ x S♀)	10	Cinnabar-a			Short 5th-3			Ragged-3		
	b (♂ x S♀)	11				Short 5th-4	Absent-4	Rough-6	Ragged-4		
Acworth, Ga. 2016.7	a (iso ♀)	5			Diminished-b						Mottled-localized
Smokemont, N.C. 2017.4	a (♂ x S♀)	Mass			Diminished-c						
	c (♂ x S♀)	17			Diminished-d	Short veins	Absent-6			Grooveless-2	
Trenton, Ga. 2018.7	a (♀ x S♂*)	12	Mahogany-4: Lustrous		Small Wing-2					Blister-3D	
Guntersville, Ala. 2019.1	a (iso ♀)	14	Cinnabar-b								Rough-missing
Hollandale, Mississippi 2021.6	a (iso ♀)	5				Semiplexus					Rough-nicked
	b (iso ♀)	2									
	c (iso ♀)	5						Rough-11			
	a (♂ x S♀)	10									Short
	b (♂ x S♀)	7	Translucent		Diminished-e						
	c (♂ x S♀)	9				Short 5th-8					
	e (♂ x S♀)	3									
	f (♂ x S♀)	3									
	g (♂ x S♀)	6			Shortened						

* Laboratory Strain of D. texana, 1128.10

vial produced *cinnabar* offspring. Five of the seven pairs of *americana* and six of the seven pairs of *texana* showed complete replacement of the mutant sperm in the second vial. The remaining pairs tested produced a few *cinnabar* individuals. These mutant individuals probably hatched from eggs which were deposited by the female before insemination by the normal male. The third vials contained only normal individuals in all cases.

The most prevalent type of mutations discovered in the *americana* populations was autosomal recessives. In addition two sex linked recessive mutants and one dominant mutation which is probably sex linked were recovered. The *roughex* mutant (2069.7, j female × b male), a sex linked recessive, was first detected among the F_1 male offspring. Crosses between F_1 females which were heterozygous for *roughex* and *roughex* males produce normal and *roughex* males and females. If heterozygous females were crossed to normal males all the female offspring and one-half the male offspring were normal. The remaining males were *roughex*. Although phenotypically similar to *echinus* of *D. virilis*, allele tests proved that *roughex* is non allelic to this mutant.

The second sex linked mutant, *yellow* (2068.6o female), is recessive and was not recovered in the F_1 generation. Allele tests to *yellow* of *virilis* have not been completed. The dominant mutant, *Abruptex* (2068.6j female × 2068.6a male), was detected in the F_3 or F_4 generation. About twelve affected females were recovered and crossed to normal males in mass matings. The ratio of *Abruptex* females to normal females to *Abruptex* males to normal males among the F_1 offspring was 1:1:0:1. The *Abruptex* male class was lethal and only fifty percent of the expected number of males was recovered. A sex linked type of inheritance or a less probable fourth chromosome linkage are indicated by the data.

The variability of the widely separated *americana* populations consisted of two mutants common to all of the tested populations and "population-specific" mutants which were found in only one or in some cases two localities. One of the widespread mutants, *light*, was recovered from every individual tested from the 2067.1 sample. The expression of this mutant was not as extreme or as frequent in the other two populations. Of the total number of 80 mutants recovered from *americana* populations, 18 were *light*.

The light mutation is probably widespread throughout the western distribution area of *americana*. The stocks retained in the laboratory from different localities of the western area have been examined and a majority of these contained lighter forms. Stocks from the eastern populations of *americana* do not exhibit such a variation of body color.

The fixation of the *light* form in at least one western population is probable. A mixture of individuals which showed light and dark body color was noticed in a stock, 1773.4e, which was established from an isolated female collected at Chadron, Nebraska, August 8, 1947. Isolation of dark and light strains was possible and further test proved that the light form was recessive. The light strains isolated, however, usually contain a low percent of intermediate gray forms. A dark strain which does not occa-

sionally produce light colored individuals has not been established. Paired matings of dark forms from the unselected stock do not produce lighter forms consistently enough or in a high enough frequency to indicate that the homozygous dark form is lethal. Cytological examination of the metaphase configuration of brain cells of dark and light strains by C. Ward showed the typical americana chromosome configuration. Further genetic test of these strains can be found in another paper in this publication.

Crosses of *light-4* (2067.1e) to *light* individuals of 1773.4e proved that the two are allelic and that the mutant has remained in the natural population at least from 1947 to 1950. These two collections, although made three years apart, were from the same locality. The first collection, 1773.4e, was made August 8, 1947 and the more recent one, 2067.1e, on August 20, 1950.

An exact determination of the frequency of *light* in the Chadron population in these two years is not possible. However, the fact that each of the eight isolated females was either heterozygous or had been fertilized by a male which carried the recessive gene heterozygous, and that two iso females (2067.1e, 2067.1h) produced F_1 individuals which were *light* suggests that the mutant reached a high frequency in the 1950 season. Stocks which have been retained in the laboratory from the 1947 collection were examined. Five of the seven stocks contained the extreme light expression and the other two showed the less extreme forms.

The expression of the light form is probably not due to a simple recessive but may depend, at least in some cases, upon modifiers or a multiple factor type of inheritance. If a multiple factor type of inheritance is involved, all of the genes for *light* are recessive to those for the dark present in the western and eastern populations. In backcross experiments which allow recombination the ratio of light to dark forms and intermediate expressions produce data beyond analysis until marker stocks can be established.

The second widespread mutant, *interrupted,* was not as frequent as *light* but the chance of morphological detection is reduced by a variation in expression. In some cases the entire posterior crossvein is missing but there are less extreme expressions, including some which approach normal. Crosses between *interrupted-8* (2068.6p) and *interrupted-9* (2069.7) produced 27 percent phenotypically affected and 73 percent normal F_1's. Test crosses between *interrupted-1* (2067.1) and *interrupted-9* gave from 22 to 80 percent affected F_1 individuals. When *interrupted-1* and *interrupted-8* were crossed, all of the F_1 offspring were normal even though each of these produced affected individuals when tested to *interrupted-9.*

The *interrupted* mutants have a very characteristic morphological expression and were recovered from three rather distant populations, the Chadron, Oakdale and Hastings. The Chadron (2067.1) and Oakdale (2068.6) samples came from areas some 240 miles apart. Hastings (2069.7) and Oakdale are about 80 miles apart; the Chadron area is separated from the Hastings sample by 260 miles. These facts suggest the widespread distribution of this mutant throughout the western *americana* populations. There are no data available for the eastern distribution range.

The "population specific" mutants of the Chadron sample are interesting in that two, *cinnabar* and *varnished,* are allelic to known mutants in the more primitive member of the group, *D. virilis.* The *cinnabar* mutant is also allelic to *cinnabar* mutants recovered in this investigation from *texana* populations. Of the remaining ten population-specific mutants recovered from the Chadron sample, five were either sterile or so abnormal in body structure that survival of homozygotes in natural populations would be impossible.

About one-half of the variations in the Chadron population were *light* or *interrupted.* The remaining variability was determined by less frequent occurring population specific mutants. An average of 1.5 mutations per fly was obtained for this sample.

The Oakdale sample of *americana* has a rather high average of 2.0 mutations per fly. The variability of this population was not limited to a concentration of one or two mutants but consisted of a low frequency of a large number of mutants. The expression of *light* mutants in this population was a low frequency of the extreme yellow form and a higher frequency of the gray variant.

A rather unusual population-specific mutant was recovered from the Oakdale population. A high percentage of individuals which showed unilateral morphological effects were recovered from two tested P_1's. The mutant, *mosaic,* was detected first by the expression of unilateral spreading of the wings. By a more thorough examination of mosaic strains, other structures such as the eyes and bristles were also found to have an abnormal, unilateral modification. There was not only a rather high percent of individuals which showed a recognizable morphological expression of the mutant but also there was a variation in the time of gene action as reflected by an effect upon different structures.

The Hastings population has the low average of 1.1 mutation per fly. About one-third of the total number of mutations recovered were *light.* The remaining population-specific mutants were autosomal recessive except for the one sex linked *roughex.*

The general structure of the western *americana* populations was composed of two consistent, widespread mutants, *light* and *interrupted,* in rather high frequency and population-specific mutants which were usually rare in occurrence and characteristic for each population. The average number of mutations per fly for the three populations was 1.69 (Table 6) which is a minimum average because a few more mutations have been confirmed since these determinations were made.

The sample of *novamexicana* was obtained from one locality, Cliff, New Mexico, and numbered eleven individuals. Gene variability of this population was very low, being 0.55 mutations per fly as shown in Table 6. The mutant distribution is shown in Table 2. All mutations were autosomal recessive and only two phenotypically similar mutants, *broken-1* and *broken-2* were recovered more than once. The *novamexicana* sample

not only differs from the *americana* and *texana* populations in the frequency of mutations but also by the absence of any mutant which effects a noticeable reduction in viability.

TABLE 6

Comparative Mutation Frequency in *Drosophila americana americana*, *Drosophila americana texana* and *Drosophila novamexicana*

Species	Total Number of Individuals Tested	Total Number of Mutations Recovered	Average Number of Mutations per Fly
Drosophila americana americana	53	80	1.69
Drosophila americana texana	107	141	1.32
Drosophila novamexicana	11	6	0.55

Samples of *texana* were taken from a greater geographic range than those of the other two species, but a great number of these populations were represented by only one or two individuals. Such small samples have been included in the study, however, since they contribute to an analysis of the population structure as a whole. The mutant distribution in *texana* populations is shown in Tables 3, 4 and 5.

The gene variability in *texana* was composed almost exclusively of autosomal recessive mutations. Only one of the 141 recovered was sex linked. This mutant, *abruptoid,* was detected in the F_1 generation of an iso female (2020.1d, Tupelo). The female was remated to a male from the same locality and again about one-half of the male offspring were *abruptoid.* About one hundred of these affected males were tested and all proved to be sterile. Dissection showed that the sterility was due to degenerate testicular development and to the absence of motile sperm.

Three species-wide mutations were found in *texana* but none of these was present in a frequency as high as those characteristic of the *americana* populations. The mutant *cinnabar,* which is a bright, scarlet-colored eye, was present in three populations which are separated from one another by a distance of from one hundred to three hundred miles. Two of the localities, Twin Lakes, Georgia, and Guntersville, Alabama, were represented by only a few individuals and the comparative gene frequencies could not be determined. A larger sample of 24 individuals collected at the third locality, Tupelo, Mississippi, did not show any concentration of

the mutant since the mutant was recoverel from one female. Crosses between the mutant from each of the three localities proved that all were allelic. These mutants were also allelic to *cinnabar* of *D. virilis*.

A second species-wide mutant, *diminished*, has several different expressions which is possibly due to an interaction of isoalleles. The extreme form is morphologically similar to dusky of *D. virilis* but it is not sex linked. The wings are about one-half the normal size with a reduction in width and length together with a darkening of the color. In the less extreme form the wings are larger and may or may not be dusky in color. In addition to these two manifestations, a third type occurs in which the ends of the wings are arched down over the end of the abdomen. Some F_1 pairs from the same P_1 parent produced one or several of these types although one type was usually more prevalent. In one case, 2015.2h, Indian Springs, the extreme form has been isolated from a strain which contained several expressions of the mutant.

Allele tests between the five *diminished* mutants, one from Indian Springs, Georgia (2015.2h), one from Acworth, Georgia (2015), one from Hollandale, Mississippi (2021) and two from Smokemont, North Carolina (2017), produced F_1 offspring which varied in the expression of the mutant. In some crosses all offspring could easily be classified as *diminished* whereas in other matings the expression varied from the extreme form to normal. In the latter cases, however, the normal individuals occurred as a low percentage of individuals.

The number of *diminished* forms detected in *texana* samples was small. In the large Indian Springs sample of 47, only one *diminished* mutant was recovered. Both of the two males in the Smokemont sample, however, produced morphologically detectable forms. The detection of this mutant was probably complicated by the interaction of iso-alleles since recovery of a pure *diminished* strain usually required selection for several generations.

A rather large number of morphologically similar mutants, *small bristles*, were recovered in three of the larger samples, the Indian Springs, Tallahassee and Tupelo localities of *texana* populations. The classification of this mutant as a species-wide mutation was complicated by the lack of allele tests since all but two of the ten recovered were completely sterile. These two, *small bristles-a* and *small bristles-b*, were allelic but were both recovered from the Tallahassee sample and thus do not furnish adequate information of the occurrence over the entire distribution range. The variation in expression of the mutant recovered from the same P_1 parent, the relatively high frequency in the Indian Springs sample and allelism of the two present in the Tallahassee sample suggest that a diverse series of alleles of this mutant exist throughout the *texana* distribution range.

The exact relation of a fourth mutant, *ragged*, has not yet been established. This mutant was not recovered in the F_2 generation but appeared in subsequent generations. Since in all but a very few cases only affected males were recovered, sex linkage was suspected and the mutant named *ragged* upon the basis of the linkage group and morphological similarity

to *ragged* of *virilis*. Further tests have established the fact that affected males are more prevalent than affected females. Whether this is due to a sexual dimorphism of the mutant, in which case an autosomal recessive type of inheritance is possible, or to a sex linked inheritance with or without an expression of sexual dimorphism has not been clarified.

Although one or two *ragged* males have been detected in a number of cases only seven mutants have been positively determined to be mutations and included in the results. These were found in the southern sector of the distribution range—Indian Springs, Georgia, Twin Lakes, Georgia, Keystone Heights, Florida, and Tallahassee, Florida. The classification of *ragged* as a species-wide mutation is still doubtful.

The gene variability of *texana* samples was composed of autosomal recessive mutations except for one sex linked mutant. There was no high concentration of a particular mutation in any of the large samples shown in Tables 3 and 4. The Indian Springs population, Table 3, showed that three-fourths of the individuals produced two, three or four mutations. No mutation was recovered from the remaining one-fourth of the sample. The number of mutations recovered in some strains may have been affected by bacterial infections.

The general structure of *texana* populations consisted of two or possibly three species-wide mutants in a low concentration and population-specific ones which were characteristic for each population. The average number of mutations per fly varied from 0.66 to 4.0 for the different samples. Some of the samples were small and the general average of 1.32 shown in Table 6 probably corresponds more nearly to the actual frequencies.

The population structure of the subspecies *americana* and *texana* is similar in that both contain widespread species-wide mutants which occur throughout the distribution range and population-specific ones which occur only in one or two samples. The gene variability of both species is primarily composed of autosomal recessive mutations. One mutation, *cinnabar*, was found to be present in both populations but occurred as a species-wide mutant in *texana* and was recovered only once in the *americana* samples.

Although species-wide mutants were recovered in both species, the concentration of these in the populations was different. The two mutants, *light* and *interrupted*, were common to all of the *americana* samples and accounted for a fairly large portion of the variability. There appears to be no particular reduction in the viability of the homozygous expression of these mutants. The *texana* populations contained no such high concentration of the characteristic widespread mutants, *cinnabar*, *diminished* and *small bristles*. Most of the *small bristles* mutants were sterile in a homozygous condition.

A comparison of *novamexicana* populations to the other two species is limited since a sample from only one locality was studied. The gene variability of this sample was lower than that for any population of the other

two species and none of the mutants recovered was noticeably below normal in viability. The only mutant similarity possible is between the *interrupted* of *americana* and *broken* of *novamexicana*. Allele tests for these two mutants have not been completed.

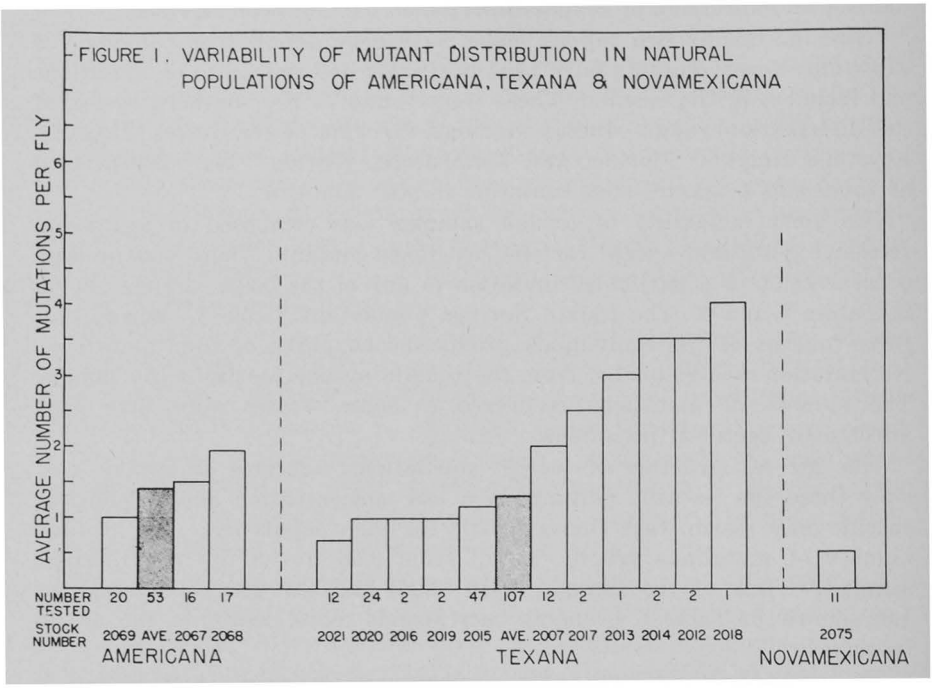


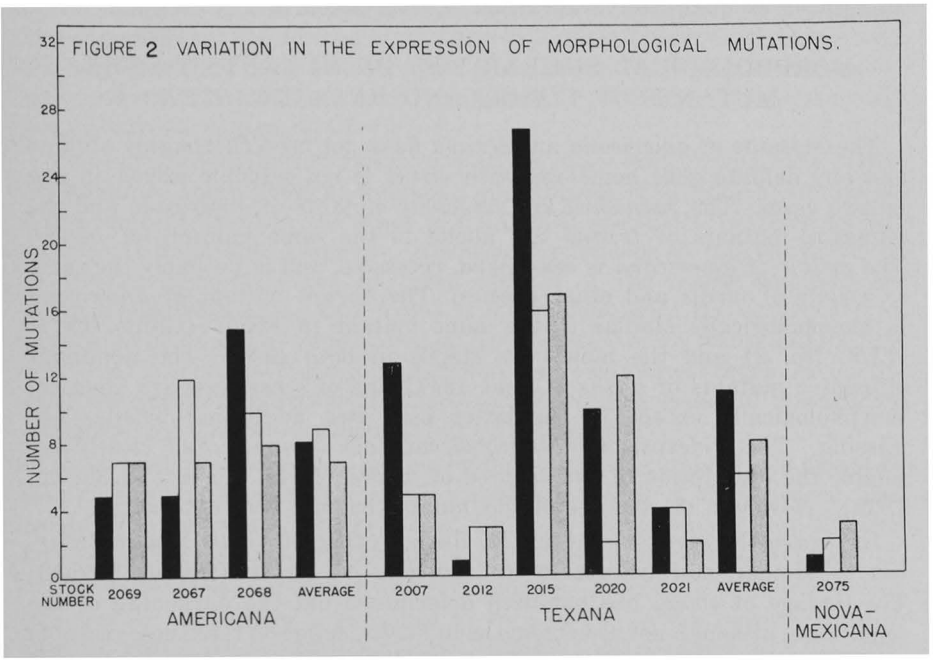
Figure 1 shows the variability for each sample of the *americana, texana* and *novamexicana* populations. The samples of each species were arranged from the less variable ones on the left to more variable ones on the right. The average for all the samples appears as a crosshatched bar and is placed in a relative position to the samples depending upon the value obtained. The number of individuals used for each determination appears under the respective sample. The average for all samples was determined from the total number of individuals tested and the total number of mutants recovered. The collection number of each sample appears below the number of tested individuals.

The *americana* samples fall slightly above and below the average but do not deviate more than about 0.5 mutations per fly. The *texana* samples show more variation but those which deviate the most are the smaller samples. The larger samples, 2007, 2015, 2020 and 2021 do not show as much relative difference. The *novamexicana* sample shows a lower value of variability than the lowest of the *texana* samples. The total averages for *americana* and *texana* samples are very similar.

The total number of individuals tested for each species, the total number of mutations recovered and the average number of mutations per

fly are given in Table 6. The averages are a measure of the minimum variability which occurred in these populations since no mutant was included unless either a stock was established, a recurrence from heterozygous mating was obtained or a relatively high percentage of morphological expression existed. The *americana* average is especially low because these samples were obtained from more recent collections and fewer generations have been obtained for thorough analysis. The *americana* populations, however, show the highest average of 1.69 mutations per fly which is fairly close to the 1.32 average obtained for *texana* populations. The *novamexicana* sample shows a low average of 0.55 mutations per fly.

A measure of more minor morphological variations as produced by multiple alleles, modifiers and possibly cumulative action of several genes is shown in Figure 2. The mutations of each sample were placed in one



of three categories. The first contains those mutants which showed a constant effect upon only one morphological structure such as bristles or wing veins. This class appears in the left column. The center column represents those mutations which had only one morphological structure affected but there was a variation in the expression of the trait. The third group consists of polymorphic mutants which show an effect upon two or more morphological structures. So few of this type of mutant showed any consistency in expression that further division of this group is impracticable. The determination of the category in which each mutant was placed was dependent upon the morphological characteristic of each

mutation. Those which had one morphological expression and were reduced in viability or sterile were placed in the first category rather than in the polymorphic one.

The *americana* populations showed a variation in the number of mutants in each category and a different pattern was produced for each sample. This is partially dependent upon the concentration of *light* and *interrupted* in the different samples. The three largest samples of *texana* populations showed a similar pattern of a high concentration of mutants in the first category and a drop in the number of those which vary in the expression of one morphological structure.

The most significant fact that is shown from this treatment of the recovered mutations is that, in all but one case (Tallahassee, 2007), the totals of the middle and right columns are greater than the first column. More than fifty percent of the mutations recovered showed either a qualitative or quantitative variation in expression.

MORPHOLOGICAL SIMILARITIES OF MUTANTS TO THE MUTANTS OF *VIRILIS* AND *MELANOGASTER*

The mutants of *americana* and *texana* have not been thoroughly studied and any definite gene homology with *virilis* is not possible except in one or two cases. The *varnished* and *cinnabar* mutants of *americana* and the *cinnabar* mutants of *texana* are allelic to the same mutants of *virilis*. The *yellow* of *americana* is sex linked, recessive, and is probably the same as *yellow* of *virilis* and other species. The *abrupt* mutant of *americana* is morphologically similar to the same mutant in *virilis* (Chino, 1934: D.I.S. No. 2) and the males are sterile in both cases. The dominant *Abruptex* mutants of *virilis* (Chino, 1941) and of *americana* are identical morphologically except in the latter case two additional bristles are missing. The widespread *interrupted* mutants of *americana* closely resemble the description of *interrupted* of *virilis* (Metz, Moses and Mason, 1923). However, the linkage of this mutant has not been determined.

In *texana* the *cinnabar* mutant is allelic to that of *virilis* and *scutellar-like* is similar morphologically to *scutellar* of *virilis* (Chino, 1936-b). The linkage of *ebony* has not been determined but the darkening of the pupal case, although not as extreme as in *virilis*, suggests that these mutants are homologous. The *bithorax, dumpy* and *grooveless* mutants of *texana* are morphologically similar to the same mutants of *melanogaster* and have not been reported in *virilis*.

DISCUSSION

Previous morphological mutation studies of natural populations of the virilis group have been limited to *Drosophila virilis* (Chino, 1936a, 1936b, 1937; Patterson, Stone and Griffen, 1942; Metz, Moses and Mason, 1923). This more primitive member differs from all other species of this group in two ways—nearly cosmopolitan distribution and domestic habitat. All

other species are limited to one zoogeographical realm and are not associated with man as are the domestic populations of *virilis*.

Cytological and genetic evidence shows that the three species, *Drosophila americana americana, Drosophila americana texana* and *Drosophila novamexicana,* are closely related and places them in one division of the virilis complex (Patterson and Stone, 1949), which is restricted to the Nearctic realm (Patterson, 1942).

Drosophila texana occurs in the southeastern portion of the United States extending as far west as central Texas and Oklahoma. The northern limit extends from North Carolina through Tennessee, Arkansas and Oklahoma. This distribution range is characterized by heavy rainfall, some swampy areas, rather heavy natural vegetation and mild to warm climate throughout the year. These conditions allow a prolonged breeding season and rather large populations are built up in some localities toward the end of the main breeding season (Patterson, 1942). The data on population size suggests that the density of *texana* populations is intermediate between the large breeding units of most *hydei* and the small populations of *limpiensis* or *novamexicana*. The yearly fluctuation of population size is probably not as extreme as that of *americana*.

The known distribution range of western *americana* includes the states of Montana, South Dakota, Nebraska and Kansas. The eastern population has a concentration in the state of Ohio with specimens reported as far west as Indiana and Arkansas. A southern extension of this range forms an overlap zone with the northern portion of the *texana* populations. Isolation between these two forms is incomplete since the occurrence of natural hybrids and of gene exchange has been proven by Stone and Patterson (1947). The short, cool summers and severe winters of this more northern distribution range probably shortens the breeding season of this species and produces greater fluctuation in population size.

Drosophila novamexicana has been found in small numbers in a distribution area which is characterized by small populations of most species. The localities from which *novamexicana* have been collected are San Antonio and Cliff, New Mexico, Whitewater, Colorado and Cave Creek, Arizona.

The basic types of morphological variation present within *americana* and *texana* populations are similar to those found in the Russian populations of *Drosophila melanogaster* by Dubinin and collaborators (Dubinin, Romashov, Heptner and Demidova, 1937); for *Drosophila immigrans* by Spencer (1940); for *Drosophila hydei* (Spencer, 1947a; Alexander, 1949); for *Drosophila limpiensis* (Alexander, 1949); and possibly for *Drosophila subobscura* (Gordon, Spurway and Street, 1939).

In each of these species two general types of morphological mutations occur. One type can be found rather widespread throughout the whole distribution range and has been designated as species-wide mutations. The second type, population-specific, includes a wide range of morphological mutants which are characteristic of one or a few localities of the distribution range.

The yet incomplete investigation of species-wide mutants of these forms makes any discussion of the general type of inheritance difficult. The *bobbed* mutants of *hydei* (Spencer, 1938), *diminished* of *texana, interrupted* of *americana* and probably *net* of *immigrans* (Spencer, 1940) exist as a complex series of iso-alleles within natural populations. One species-wide mutant present in populations of *subobscura* (Gordon, Spurway and Street, 1939), *striped* in *hydei* and *Mottled* in *limpiensis* (Alexander, 1949) showed a weak dominance often affected by gene modifiers. The *Mottled* mutant type may depend upon multiple factors. The widespread *light* mutants which were found in the western *americana* populations are recessive with different intensities of expression in the three different populations. The *cinnabar* mutants of *texana* are recessive with no apparent variation in expression.

Population studies reflect the success of certain mutants to become widespread and thus the more important in evolution as an extension of the genetic variability. About one-half of the wide-spread mutants which have been reported exist as a complex series of iso-alleles within natural populations. The *bobbed* mutants of *hydei* and *net* mutants of *immigrans* have been reported to be iso-alleles by Spencer (1938, 1940). The morphological expression of the species-wide mutant, *trident,* which has been found in Russian populations of *melanogaster* (Dubinin and collaborators, 1937) suggest the possibility that a series of iso-alleles exist. The *diminished* mutants of *texana* and *interrupted* mutants of *americana* also exist as a series of multiple alleles within natural populations.

The efficiency of this type of inheritance should not be overemphasized since other types of mutants have most certainly become widespread and fixed in populations. The *light* mutant has been up to the present time more successful, if success be based upon frequency, in the Chadron sample than the *interrupted* mutants.

Apparently the attainment of widespread distribution of any mutant throughout a species range is not dependent upon any one type of inheritance. A fairly mutable locus and a slight or high selective advantage are characteristics which will allow such genes to become widespread. The retention and concentration of these mutants in different populations probably depend more upon population dynamics and fluctuations.

A difference in the concentration of *light* (species-wide) in the Chadron population of *americana* and the other two localities exemplifies the results of such population dynamics. A moderately high level of this mutant has apparently been stabilized in this population whereas the other populations tested contained a lower frequency of the mutant and less extreme morphological forms. This particular population is rather isolated, thus preventing much interbreeding with other populations and most likely undergoes a sharp bottleneck in population size during the winter. Several general paths by which such a frequency could be attained lie within the realm of probability. Linkage with genes with a selective advantage or genetic drift and chance fixation seem to be the more likely possibilities in this case.

Spencer (1944) tested two populations of *hydei* for the frequency of a species-wide mutant, *bobbed*. One locality, Azusa, Southern California, contained a large population (estimated to be composed of 100,000,000 individuals) of *hydei* breeding in a citrus dump. The other population tested was from Wooster, Ohio, and was estimated to be of a magnitude of not less than 500,000 in size. By crossing phenotypically normal females of these populations to a standard stock, Wooster 20, Spencer obtained a high concentration of a few grades of bristle sizes and less spread in the allelic series from the Wooster sample than from the Azusa population. The population size and year-round pattern was used as an explanation for the difference in the *bobbed* frequency in these two populations.

Apparently not only species-wide mutants but also population-specific ones may attain a high frequency within a population. Spencer (1947b) reported a high frequency of the mutant, *stubble*, in a *D. immigrans* population. There was no apparent concentration of population-specific mutants in the *texana, americana* or *novamexicana* populations. In general the population-specific mutants had a lower frequency than species-wide ones in the *americana* populations. The *texana* samples showed no discrepancy in the frequency of population-specific and species-wide mutations.

There was such a tremendous amount of diversity in the category of population-specific mutations recovered from the populations studied in this investigation that a discussion of each would be impossible. However, a reduction in viability or fertility was characteristic for most mutant types.

The unusual action of one mutant, *mosaic*, which was recovered from the Oakdale collection of *americana* warrants discussion. The expression of this mutant is usually unilateral with a more frequent action upon the wings, eyes and bistles. A rather high percentage of individuals with detectable morphological changes in isolated strains of the mutant, and the absence of such phenotypic variation in other strains tested from this and the other two populations, strongly suggest that environmental conditions such as temperature and moisture are not responsible for the morphological changes. The detection of two sex-linked mutants, the recessive *yellow* and dominant *Abruptex*, as well as the high mutation average of 2.0 for this population may be entirely independent of the presence of the *mosaic* mutant. The effect of *mosaic* upon different structures suggest, however, a variation in the time of gene action. Such variation in the time of gene action need not be restricted to a period which produces the individuals classified as *mosaic*.

Sex-linked mutations in natural populations are rather rare when compared to those which show autosomal linkage. The action of selection is not as slow since the homozygous condition of the sex chromosome in the male allows the same selective action as the homozygous, autosomal condition. One exception to such a system of selection is the sex-linked mutants at the *bobbed* locus which have normal or less extreme alleles in the Y chromosome. In this case selective action is reduced even below that of autosomal recessives if the Y chromosomes contain equally normal alleles. Since

selection usually operates more efficiently upon a homozygous condition, the action is thus limited to females which are further protected in this particular case by a series of interacting iso-alleles. The presence of iso-alleles of *bobbed* in natural populations of *hydei* has been investigated by Spencer (1938, 1944). He found that this mutant reached such a distribution maximum that it can be considered a species-wide mutant, as it is common to most populations over the whole distribution range. Different populations were found to contain different concentrations and frequencies of this complex series of iso-alleles. Parallel mutations have been recorded in *D. melanogaster* (see Bridges-Brehme, 1944); in *D. simulans* (Sturtevant, 1929); in *pseudoobscura* (Sturtevant and Tan, 1937); in *affinis* (Sturtevant, 1940); in *ananassae* Moriwaki, 1935: Kikkawa, 1938); in *subobscura* (Jermyn, Philip, Rendel and Spurway, 1943, D.I.S. No. 17); in *funebris* (Timofeeff-Ressovsky, 1931; Spencer, 1934, D.I.S. No. 2); and in *virilis* (Chino, 1936b).

Although this sex-linked mutation has been found rather wide-spread throughout the genus Drosophila including *virilis*, no proven case was found in *americana*, *texana*, or *novamexicana*. Nevertheless, three and possibly four mutations, which showed a simple sex linkage, were recovered from *americana* and *texana*.

The sex-linked, recessive mutation, *abruptoid*, was carried by an iso female collected from the Tupelo population of *texana*. The F_1 male offspring were normal or *abruptoid* which is expressed morphologically as a shortening of the longitudinal veins of the wings. A strict 1:1 ratio was not obtained because of the reduced viability of the mutant. The fact that all affected males were completely sterile suggests a recent origin for this mutant since selective action against such a mutation should rapidly eliminate it from a population.

In *americana* two sex-linked recessives and one dominant mutation which is probably sex-linked were recovered. The dominant mutant, *Abruptex* (2069, Oakdale collection), either occurred as a spontaneous mutation in a phenotypically normal fly or was masked by some type of supressor gene or genes. Affected females crossed to normal males produce one-half normal females and one-half *Abruptex* females. All the F_1 males are normal and occur in about one-half the expected male frequency, thus proving a lethal action of the gene in males. Such lethal expression in males suggests a sex-linkage since the homozygous condition of the sex chromosome is more apt to allow a detrimental action than an autosomal heterozygous condition. The possibility of the presence of the gene in the male without expression is reduced by the recovery of only one-half of the expected number of males. Such reduction in number could occur if an unrelated sex-linked lethal was present in an *Abruptex* female but the test matings were made in mass and not in pairs. If one or even two of the six *Abruptex* females carried a lethal in one of the sex chromosomes, there would have been a decrease in the number of males, but not as definite a ratio in the reduction would have been obtained. Another possible but less probable explanation is the assumption of a fourth chromosome linkage.

In this particular species an X-4 chromosome fusion (Patterson, Stone and Griffen, 1940) permits recessive mutations in the fourth linkage group to show sexual dimorphism since a free fourth chromosome is present in the males and is inherited from male to male in the same way as the Y chromosome. In this case the lethal effect in the males would require the further assumption that the *Abruptex* gene in the free fourth chromosome will always interact with the allele carried by the female to produce a lethal action, or that it exhibited a special type of sex limited lethality which is improbable.

A rough eye mutant, *roughex*, which is a sex-linked recessive was detected in the F_1 male offspring of a cross between the j female and b male of the Hastings collection of *americana*. The comparative lack of a reduction in the viability of this mutant, as could be measured by the ratio of *roughex* to normal males, does not necessitate an assumption of recent origin for this mutant. Such a possibility can not be eliminated entirely since only one female of the twenty tested individuals carried this mutant.

The second sex-linked recessive mutation, *yellow*, was recovered from the Oakdale population. This mutation could have occurred as a spontaneous mutation in some subsequent generation after collection of the parent, since none of the F_1 offspring of the iso female was affected. In this case, however, the presence of a sex-linked lethal or semi-lethal would reduce the number of affected males and delay detection of the mutant until crossing over and recombination allowed a more suitable combination of genes. The allelism to *yellow* of *virilis* has not yet been tested but the phenotypic expression of this mutant is very similar to *yellow-40* of *virilis*.

Among the sex-linked mutations recovered from populations of Drosophila species, *yellow* has been one of the more common types. Different frequencies of *yellow* in Russian populations of *melanogaster* have been reported by Dubinin and collaborators (1937) and Berg (1942a, 1942b). The recovery of yellow individuals in American strains of *melanogaster* has been reported by Spencer (1944). Metz found one yellow male in a collection of *simulans* (Sturtevant, 1929). A sex-linked, recessive *yellow* mutant in *immigrans* was first reported by Stella (1936) and *yellow* individuals among wild specimens of *immigrans* have been found by Spencer (1944).

The amount of morphological variability concentrated as sex-linked mutations is very small in *americana* and *texana* populations as in other Drosophila species which have been investigated. The detection of few mutants with a simple sex-linked type of inheritance in natural populations is important since the occurrence of spontaneous mutations in the sex chromosome can be proven and the reduction in number conforms to the general theory of selection. Such a mutation as *bobbed* is a special case in which two adaptive mechanisms have been developed.

Major morphological mutants form only a small portion of the total variability of natural populations. Detectable lethal mutations have been estimated to be five to ten times more frequent than visibles by Spencer (1947a).

Dobzhansky and Wright (1941) found that the lethal mutation rate in the third chromosomes of *pseudoobscura* which were collected from Guatemala, Mexico and Death Valley were substantially the same (Death Valley 0.0027 ± 0.00032, Mexico 0.00359 ± 0.00062, Guatemala 0.00284 ± 0.00061), but 15.29 ± 0.83 percent of the chromosomes tested from Death Valley carried one or more lethals. In similar samples from Mexico and Guatemala, values of 28.1 ± 3.3 percent and 34.2 ± 5.2 percent were obtained. The difference of lethal concentration in this case was apparently not due to mutation rate but to some other factor or factors, presumably population size and selection pressure.

Differences between the Russian and American populations of *melanogaster* have been shown by the work of Dubinin (1946) and Ives (1945). Dubinin found mutation rates of 0.33 ± 0.007, 0.44 ± 0.08, 0.45 ± 0.1 percent for the second chromosome extracted from three different Russian populations. Ives obtained lethal mutation rates of from 0.49 to 6.20 percent for the same chromosome in American populations from different localities. One explanation for such discrepancy in the mutation rates is offered by the presence of genes which increase the mutation rate. Some Florida strains of *melanogaster* have been found to contain such "mutators" by Ives (1950). If homozygous, the action of this gene increases the mutations in many genes more than ten times the normal. The effect upon the rate of different genes varies, however. The mutation rate of the *folded* mutant was increased more than any other gene tested whereas *yellow* is not increased.

Additional data on the frequency of allelic mutations in American and Russian populations show that the American *melanogaster* breeds in comparatively large populations, and that these populations are continuous from year to year in the tropical, sub-tropical and temperate zones of the United States (Ives, 1945). The high concentration of a few mutants, both visibles and lethals, in Russian populations seems to indicate that these populations underwent a sharp reduction at one or more seasons of the year and then expanded in size (Spencer, 1947a).

The difference of genetic structure of populations of this and other species may be real or only superficial. Discrepancy may be obtained by the interpretation of different workers and different methods of procedure. Sampling at different times in the seasonal cycle as reflected in population size could certainly give quite different estimates. The male to female ratio in the samples of *americana* and *texana* suggests that these populations might have been at a different point in such a seasonal cycle. The *texana* samples were collected in June and were composed primarily of males. The *americana* collections were made in August and contained a predominance of females. A difference in the frequency of species-wide mutants within the populations of these two species may have been partially due to sampling at some point in the cycle. The pattern of the seasonal cycle—that is the time and extent of population peak and reduction —could, however, explain such differences. We do not know if the males

and females of the two species differ in their response to bait or in motility.

Certain physiological features of a species can possibly also determine the amount of gene variability which can be detected. Physiological differences, as reflected by the number of phenocopies obtained, exist between *hydei* and *limpiensis* at 22 degrees Centigrade (Alexander, 1949). The increased amount of crossing over in *virilis* and apparent inefficiency of simple inversions as balancers would allow more gene recombination and genotypes for the detection of morphological mutants than in *melanogaster*.

The treatment of morphological variations which are present within natural populations is difficult in that a graded series of intermediates between an easily classified mutant type and normal exist. This characteristic is inherent within the genetic system and it would indeed be surprising not to find a series of variations of different levels of divergence.

The complexity of morphological expression has been ably demonstrated by Timofeeff-Ressovsky (1934a, 1934b). The effect of more minor cumulative differences which have no particular morphological expression has been tested in *D. pseudoobscura* (Dobzhansky and Spassky, 1944 and Dobzhansky, Holz and Spassky, 1942) and in *D. Funebris* (Timofeeff-Ressovsky, 1935). All these data show not only a complex reaction between the genetic system and the external environment, such as temperature, but also an interaction between minor changes within the genetic system itself. Figure 2 shows the variation in the expression of morphological mutations in the *texana, americana* and *novamexicana* populations tested.

Natural mutation studies of this type measure the results of a number of interacting factors in the general process of evolution. The assignment of any one factor or factors in any one case is difficult and requires extensive study of the particular mutant, the species concerned, environmental conditions, population size and fluctation. Population studies have revealed, however, that a great amount of variability exists . This variation may be in the form of chromosome aberrations (fusions and inversions), gene mutation and minor variants. It is obvious that such variation is not incidental nor independent of the general process of evolution but is a part of that process.

SUMMARY

1. Natural populations of three closely related species, *Drosophila americana americana, Drosophila americana texana* and *Drosophila novamexicana,* were tested for morphological variation. Populations of *americana* from three localities, populations of *texana* from eleven localities and one *novamexicana* population were used.

2. The medium-sized populations of *americana* and *texana* showed a 1.69 and 1.32, average of mutations per fly, respectively. A low average of 0.55 was obtained for the *novamexicana* population.

3. The basic mutation structure of *texana* and *americana* were similar in that both contained two general types of mutation, species-wide and population-specific. The *cinnabar* and *diminished* mutants were found in three or more widely separated populations of *texana*. Different alleles of *diminished* interact to give a variation in expression and are therefore classified as iso-alleles. The *cinnabar* mutants give only one detectable expression. Two wide-spread mutants, *interrupted* and *light*, were found in all three populations of western *americana*. The *interrupted* mutants act as iso-alleles while *light* is recessive with modifiers. The two species-wide mutations of *americana* showed a higher gene frequency in the tested populations than *cinnabar* and *diminished* of *texana*.

4. The *cinnabar* mutant was recovered from *americana* and *texana* populations but occurred as a species-wide mutant in the latter and only once in the former. The *varnished* mutant of *americana* and *cinnabar* of *texana* are allelic to similar mutations in *virilis*.

BIBLIOGRAPHY

Alexander, M. L. (1949). Note on Gene Variability in Natural Populations of Drosophila. The Univ. of Texas Publ. No. 4920 : 63–69.

Berg, R. L. (1942a). Mutability as Dependent on the Degree of Isolation of Population of *Drosophila melanogaster*. C. R. (Doklady) Acad. Sci. USSR, N.S. 36: 71–75.

Berg, R. L. (1942b). Mutation Rate in Populations of *Drosophila melanogaster* Inhabiting Boundary Areas of the Species Distribution. C. R. (Doklady) Acad. Sci. URSS, N.S. 36: 154–159.

Bridges, C. B. (K. S. Brehme, editor) (1944). The Mutants of *Drosophila melanogaster*. Carn. Inst. Wash. Publ. 552 : 1–252.

Chino, M. (1936a). The Genetics of *Drosophila virilis*. The Jap. Jour. of Genet., Vol. XII, No. 4 : 187–210.

Chino, M. (1936b). The Genetics of *Drosophila virilis*. The Jap. Jour. of Genet., Vol. XII, No. 5 : 257–277.

Chino, M. (1937). The Genetics of *Drosophila virilis* (III). The Jap. Jour. of Genet., Vol. XIII, No. 2 : 100–120.

Chino, M. (1941). New Mutants in *Drosophila virilis virilis* (1) The Jap. Jour. of Genet., 17 : 185–206.

Dobzhansky, Th. and Epling, C. (1944). Contributions to the Genetics, Taxonomy, and Ecology of *Drosophila pseudoobscura* and Its Relatives. Carne. Inst. Wash. Publ., 554 : 1–183.

Dobzhansky, Th., Holz, A. M. and Spassky, B. (1942). Genetics of Natural Populations VIII. Concealed Variability in the Second and Fourth Chromosome of *Drosophila pseudoobscura* and Its Bearings on the Problem of Heterosis. Genetics 27 : 463–490.

Dobzhansky, Th. and Spassky, B. (1944). Genetics of Natural Populations XI. Manifestation of Genetic Variants in *Drosophila pseudoobscura in* Different Environments. Genetics, 29 : 270–290.

Dobzhansky, Th. and Wright, S. (1941). Genetics of Natural Populations V. Relations between Mutation Rate and Accumulation of Lethals in Populations of *Drosophila pseudoobscura*. Genetics, 26 : 23–51.

Dubinin, N. P., Romashov, D. D., Heptner, M. A., and Demidova, Z. A. (1937). Aberrant Polymorphism in *Drosophila fasciata* Meigen. Biol. Zh. Mosk. T. VI, No. 2 : 311–354.

Dubinin, N. P. (1946). On lethal Mutations in Natural Populations. Genetics, 31 : 21–28.

Gordon, C., Spurway, H., and Street, P. A. (1939). The Analysis of Three Wild Populations of *Drosophila subobscura*. Jour. of Genet., 38 : 37–90.

Ives, P. T. (1945). The Genetic Structure of American Populations of *Drosophila melanogaster*. Genetics, 30 : 167–196.

Ives, P. T. (1950). The Importance of Mutation Rate Genes in Evolution. Evolution, Vol. IV, No. 3 : 236–296.

Kikkawa, H. (1938). Studies on the Genetics and Cytology of *Drosophila ananassae*. Genetica, 20 : 458–516.

Metz, C. W., Moses, M. S., and Mason, E. D. (1923). Genetic Studies on *Drosophila virilis* with Considerations on the Genetics of Other Species of Drosophila. Carne. Inst. Wash. Publ., 328 : 1–94.

Moriwaki, D. (1935). Bobbed Mutations in *Drosophila ananassae*. Proc. Imper. Acad. of Japan II : 340–341.

Patterson, J. T. (1942). Distribution of the Virilis Group in the United States. Univ. of Tex. Publ., No. 4228 : 153–161.

Patterson, J. T., Stone, W. S., and Griffen, A. B. (1940). Evolution of the Virilis Group in Drosophila. Univ. of Tex. Publ. No. 4032 : 218–250.

Patterson, J. T., Stone, W. S., and Griffen, A. B. (1942). Genetic and Cytological Analysis of the Virilis Species Group. Univ. of Tex. Publ., 4228 : 162–200.

Patterson, J. T., and Stone, W. S. (1949). The Relationship of *novamexicana* to the Other Members of the Virilis Group. Univ. of Tex. Publ., 4920 : 7–17.

Spencer, W. P. (1938). Multiple Alleles at the Bobbed Locus in Populations of *Drosophila hydei*. Genetics, 23 : 170.

Spencer, W. P. (1940). On the Biology of *Drosophila immigrans* Sturtevant with Special Reference to the Genetic Structure of Populations. Ohio Jour. of Sci., XL, No. 6 : 345–361.

Spencer, W. P. (1944). Iso-allels at the Bobbed Locus in *Drosophila hydei* Populations. Genetics, 29 : 520–536.

Spencer, W. P. (1947a). Mutations in Wild Populations in Drosophila. Advances in Genetics, Vol. I : 359–402.

Spencer, W. P. (1947b). Genetic Drift in a Population of *Drosophila immigrans*. Evolution I : 103–110.

Stella, E. (1936). Etudes Genetiques et Cytologiques sur *Drosophila immigrans* Sturtevant. Rev. suisse Zoology 43 : 397–414.

Stone, W. S. and Patterson, J. T. (1947). Species Relationships in the Virilis Group. Univ. of Tex. Publ. 4720 : 157–160.

Sturtevant, A. H. (1929). Contributions to the Genetics of *Drosophila simulans* and *Drosophila melanogaster* I. The Genetics of *Drosophila simulans*. Carne. Inst. Wash. Publ., 399 : 1–62.

Sturtevant, A. H. (1940). Genetic data on *Drosophila affinis*, with a Discussion of the Relationships in the Subgenus Sophophora. Genetics, 25 : 337–353.

Sturtevant, A. H., and Tan, C. C. (1937). The Comparative Genetics of *Drosophila pseudoobscura* and *Drosophila melanogaster*. Jour. of Genet., 34 : 415–432.

Timofeeff-Ressovsky, N. W. (1931). Zur Genetik der *Drosophila funebris* I. Ge schlechtsgebundene Vererbung. Arch. Entw. Mech. Org., 124 : 154–180.

Timofeeff-Ressovsky, N. W. (1934a). Über die Vitalität einiger Genmutationem und Ihrer Kombinationen bei *Drosophila funebris* und Ihrer Abhangigkeit vom "genotypischen" und vom äusseren Milieu. Zeit. Ind. Abstam, und Vererbungs., Bd. LXVI : 319–344.

Timofeeff-Ressovsky, N. W. (1934b). Über den Einfluss des Genotypischen Milieus und der Aussenbedingungen auf die Realisation des Genotyps. Nachrichten von der Gesellschaft der Wissenschaften zu Göttingen, Bd. 1, Nr. 6 : 53–106.

Timofeeff-Ressovsky, N. W. (1935). Über Geographische Temperaturrassen bei *Drosophila funebris*. Arch. Naturgesch, N.F. 4:245–257.

Timofeeff-Ressovsky, N. W. (1940). Mutations and Geographical Variations : 73–136. The New Systematics, Oxford at the Clarendon Press.

APPENDIX

The mutations which were recovered from natural populations of *americana, texana* and *novamexicana* have been given names and listed alphabetically. The mutants for the three species are included as separate lists. The mutant name is followed by the collection number of the stock, the type of inheritance and a brief description of the mutant. Double names, although usually not desirable, were assigned to those mutants which would be of no further use in genetic work. This conserves the more specific names.

Drosophila americana americana

1. *abrupt;* 2069; autosomal recessive. All of the longitudinal wing veins are shortened and the ocellar and/or orbital bristles are missing. Males sterile.

2. *Abruptex;* 2068; dominant, probably sex linked, lethal in the male. All of the longitudinal veins are shortened to about one-fourth the normal length. The posterior crossvein or a part of the vein is always present. The wings are wide across the center portion and fold under along the edges. There is a reduction in the size of the eye and the normal curvature is absent. The facets show complete abnormal arrangement and the pile is short. An absence of the orbital, ocellar, postvertical, presutural, posterior notopleural and supra-alar bristles is characteristic. Females are fertile. Morphologically similar to *Abruptex* of *virilis* (Chino, 1941).

3. *arched;* 2068; autosomal recessive. The wings are rolled under along the edges and arched down over the abdomen.

4. *aristapedia-like;* 2069; autosomal recessive. The aristae are thickened and often segmented. The aristal hairs are always present although reduced in number. Claw-like structures are sometimes present on the distal end of the aristae. There is no reduction in the length of the bristles to insure that this is a *spineless-aristapedia* allele.

5. *blunt;* 2068; autosomal recessive. The bristles are reduced in diameter and slightly increased in length. In older flies the distal half of the bristles breaks off thus appearing short and stubby.

6. *bubble;* 2069; autosomal recessive. Thin, puffed spots of different sizes occur in the marginal or submarginal wing cell. The wings are brown and curled but when expanded are narrow in width and pointed on the ends.

7. *cinnabar;* 2067; 3rd chromosome; autosomal recessive. The eye is a bright orange which is retained after aging. Allelic to *cinnabar* of *texana* and *virilis*.

8. *constricted;* 2069; autosomal recessive. The wings are short and narrow with rounded ends.

9. *constricted-like;* 2068; autosomal recessive. Phenotypically similar to *constricted.*

10. *displaced;* 2068; autosomal recessive. The eyes have displaced, swollen and occasionally fused facets. The scutellar, dorso-central, humeral and ocellar bristles are missing. The wings contain enlarged cells and patches of abnormally arranged cells which give it a pebbly appearance.

11. *double;* 2067; autosomal recessive. The anterior scutellar bristles are doubled. The expression is usually unilateral.

12. *downcast-sterile;* 2067; type of inheritance not determined. The wings are folded downward parallel to the thorax and are often folded under the thorax. Only affected females which showed no ovary development were recovered.

13. *everted;* 2067, 2069; autosomal recessive. A large mass of undifferentiated tissue extends from the external opening of the digestive tract. In the males the external genitalia may be normal in appearance, rotated to any extent and in any direction or missing entirely. The males have only rudimentary testes which contain sperm bundles but no motile sperm. The spermathecae are present in the females. No other normal structures of the reproductive system are present. The digestive tract of both males and females end within the abdominal cavity as an unattached gut.

14. *extreme-1;* 2068; autosomal recessive. The bristles are reduced in length and diameter. Any of the bristles of the head or thorax may be missing. Extra wing veins from the second longitudinal wing vein to the marginal vein sometime occur.

15. *extreme-2;* 2069; type of inheritance not determined. All of the bristles are reduced in length and diameter and any one or more may be missing entirely. The eyes contained fused and displaced facets. Only affected females recovered.

16. *extreme-like;* 2067; autosomal recessive. The bristles are reduced in length and diameter. In the females there is no hair growth or pigmentation on the abdomen; the males show almost normal pigmentation with sparse hair growth.

17. *hairless;* 2067; autosomal recessive. The number of hairs on the thorax is reduced to about one-half the normal number. Occasionally the bristles may also be missing.

18. *grooveless-like;* 2068; autosomal recessive. The line of demarcation between the thorax and scutellum is obliterated.

19. *immature;* 2069; autosomal recessive. The abdomen is immature in appearance.

20. *increased;* 2069; autosomal recessive. The eye facets are somewhat larger than normal and show abnormal arrangement.

21. *interrupted;* 2067, 2068, 2069; autosomal recessive. The posterior crossvein may have a small gap in the center, one-half of the vein or the entire vein may be missing. One or both posterior crossveins may be affected.

22. *light;* 2067, 2068, 2069; autosomal recessive with modifiers. The body pigmentation is yellow or gray as compared to the darker pigmentation of normal flies. The hairs and bristles are normal in color. There is a variation in expression of the mutant in different strains.

23. *mosaic;* 2068; type of inheritance not determined. The gene expression is a unilateral one affecting several different structures of the body. One wing is usually spread in a horizontal plane. Other unilateral effects are rough eye, extra wing veins, cut wings and missing bristles.

24. *narrow;* 2068; autosomal recessive. The wings are narrow and pointed. The wing cells are larger than normal.

25. *orange;* 2068; autosomal recessive. The eye is translucent and orange in color. The color is bright upon emergence and darkens upon aging.

26. *parallel;* 2068; autosomal recessive. The body pigmentation is a straw yellow. The wings are narrow, straight along the edges and taper to a point.

27. *pointed;* 2069; autosomal recessive. The wings are narrow, slightly longer than normal and pointed. Complete separation from normal is difficult.

28. *red;* 2067; autosomal recessive. The eye is translucent and an orange-red in color.

29. *rough;* 2069; type of inheritance not determined. The eye is rough due to abnormal pile development. Only affected female recovered.

30. *roughex;* 2069; sex linked recessive. The eye facets show slight disarrangement but the rough appearance is produced primarily by abnormal pile arrangement. Non-allelic to *echinus* of *virilis.*

31. *rough-cut;* 2067; type of inheritance not determined. The eye facets are abnormal in arrangement, fused and swollen. The longitudinal fourth and fifth veins are constricted through the middle portion to form a thickened junction with the posterior crossvein. The wings are small, short and notched. The wings are sometimes spread and some of the thoracic bristles may be missing. Only females were recovered which suggest either a lethal or balanced condition in the males.

32. *rough-spread;* 2069; autosomal recessive. The eyes are rough and one or both wings may be spread from 45 to 90 degrees. The wings have a brownish tinge and extra wing veins extend from the second and third ligitudinal wing veins. A vesiculated area in the distal half of the wing occurs. Males and females show rudimentary internal reproductive organs.

33. *slight;* 2067, 2068; autosomal recessive. The bristles are reduced in length and diameter. The eyes have abnormal facet arrangement and the pile is reduced in length thus giving the eye a smooth appearance. The entire fly is smaller in size and lighter in color.

34. *small bristles;* 2068; autosomal recessive. The bristles are reduced in length and occasionally in diameter. Sterility of this mutant is due to the degenerate ovary development in the female and absence of motile sperm in the male.

35. *spread;* 2068, 2069; autosomal recessive. Both wings are spread from 45 to 90 degrees in a horizontal plane.

36. *strand;* 2068; autosomal recessive. The scutellar and dorso-central bristles are reduced to a small strand. Variation in expression and overlaps normal.

37. *swollen;* 2068; autosomal recessive. The tarsal joints are shortened and a swollen knot appears on the first or second tarsal segment.

38. *tapering;* 2067; autosomal recessive. The wing edges are somewhat parallel and taper to a point on the end.

39. *tinted;* 2068; autosomal recessive. The wings are wide with rounded ends and slightly dusky in color. Males and females are sterile.

40. *varnished;* 2067.1b; autosomal recessive, Chromosome 2. The eye is almond-shaped and the facets are fused in such a way to produce a glossy appearance. The eye color is orange but a large colorless spot in the center of the eye is sometimes present. The ocelli are pale yellow. This mutant is allelic to *varnished* of *virilis.*

41. *weak;* 2068; autosomal recessive. Pleiotropic mutant which produces abnormal phenotypic expression of the wings, eyes, legs and bristles. The fly is weak and often dies almost immediately after emergence from the pupal case.

42. *wide;* 2068; autosomal recessive. The 3rd posterior wing cell is wider than normal and the outer edge constricts at the distal termination of the fifth longitudinal wing vein. Complete separation from normal is difficult.

43. *wine;* (1–3); 2068, 2069; autosomal recessive. The eye is opaque and a deep ruby-red in color. No allele tests.

44. *yellow;* 2068; sex linked, recessive. The body pigmentation is yellow being about the same color as *yellow 40a* of *virilis.* All the microchaetae of the body and wings are yellow as is the pile of the eye. The distal half of the bristles and elements of the aristae contain yellow pigmentation.

Drosophila novamexicana

1. *broken* (1–2); 2075a, 2075b; autosomal recessive. The posterior crossvein may be missing entirely or only a small section of the vein lost.

2. *curved;* 2075.8b; autosomal recessive. The wing edges are curved under along the entire length of the wing and the ends are arched down over the end of the abdomen.

3. *shaggy;* 2075.8c; autosomal recessive. The eyes have a rough appearance and most of the hairs of the body are irregular in the direction of growth. They may be directed dextral, sinistral or perpendicular to the normal hair growth direction. The tarsal joints are shortened and appear to have a heavier growth than normal.

4. *shortened;* 2075.8h; autosomal recessive. The wings are small, short and taper to a point. The posterior crossvein may be gapped or missing entirely.

5. *sparse;* 2075.8f; autosomal recessive. Areas occur on the thorax, eyes and wings which are completely denuded of microchaetae and macrochaetae. Other variations as extra wing veins and notched wings may occur.

Drosophila americana texana

1. *abnormal abdomen-1,* -2; 2015; autosomal recessive. The tergite formation is abnormal although there is no reduction in the number of abdominal segments present. The whole abdomen shows an almost complete loss of pigmentation and only a few hairs are present.

2. *abnormal abdomen-2;* 2020; autosomal recessive. The abdomen shows some loss of hairs, bristles and pigmentation. The eyes show some abnormal arrangement of facets and the pile is missing in spots.

3. *abruptoid;* 2020.1d; sex linked, recessive. All the longitudinal wing veins are shortened. Males are sterile.

4. *absent* (1–7); 2007, 2012, 2014, 2015, 2017; probably autosomal recessive. Any bristles of the head or thorax may be missing. The scutellar bristles are most often affected. No allele tests have been completed.

5. *absent-semi-lethal;* 2015; autosomal recessive. Various bristles of the head and thorax are missing. The sclerite formation is abnormal with a mosaic pattern of nonpigmented patches. Extra wing veins may branch from the longitudinal veins or appear as isolated fragments. Late emergence and low viability.

6. *bithorax;* 2007.1d; autosomal recessive. An extra growth of tissue is present between the thorax and abdomen. The halteres are enlarged, abnormal in shape and bent downward. The tibia is knotty and the tarsal segments are shortened. One wing is sometimes spread.

7. *blister;* 2013.1a; autosomal recessive. A thin area of the wing at, or near, the region of the anterior crossvein produces a blistered spot.

8. *blister-3d;* 2018.1a; autosomal recessive. A small area of thin wing tissue in the 3rd posterior wing cell produces a bubble-like structure. The expression is limited to this area.

9. *bright-1;* 2020.1a; autosomal recessive. The eye is bright orange upon emergence but darkens to nearly normal in two or three days.

10. *bright-2;* 2020.1e; autosomal recessive. The eye is translucent and bright orange-red in color. Darkens to nearly normal upon aging.

11. *brilliant;* 2015; autosomal recessive. The eyes are translucent and bright orange-red. There is some slight darkening when aged. Non allelic to *scarlet, cinnabar* and *cardinal* of *virilis.*

12. *cinnabar;* 2014, 2019, 2020; autosomal recessive, Chromosome 3. The eye is a bright orange in color. Allelic to *cinnabar* of *virilis.*

13. *closed;* 2007; autosomal recessive. The external genitalia and the external openings to the reproductive and digestive tracts are absent in both male-like and female-like individuals.

14. *diminished;* 2015, 2016, 2017, 2021; autosomal recessive. These mutants have three expressions which are probably dependent upon multiple iso-alleles. The extreme form is similar to *miniature* in that the wings are reduced in size and dusky in color. In the less extreme form the wings are not as small and may or may not be dusky in color. In the third form the wings are arched down over the end of the abdomen. A pure strain showing only one expression has been isolated from a mixed culture which showed several. All five *diminished* mutants are allelic.

15. *dishevelled;* 2015; autosomal recessive. Some eye facets are swollen and/or show abnormal arrangement. One or both wings may be spread in a horizontal plane. The tarsal joints are shortened and extremely long hairs and bristles are present in this region. The macrochaetae and microchaetae of the thorax are abnormal in growth arrangement.

16. *downcast;* 2015; autosomal recessive. The wings are sagged downward at a 25–45 degree angle to the body. The male, female or both are sterile.

17. *dumpy;* 2015; autosomal recessive. The wings are about one-half the normal length and broad. The posterior half of the wing is missing. Rather dark, heavy hair growth occurs along the cut edges. All veins of the remaining portion of the wing are normal. Whorls of hair on each side of the thorax sometimes occur. Males and females reduced in viability and probably sterile.

18. *ebony;* 2020; autosomal recessive. The entire body is darkened by black pigment. The eyes are also darker than normal. The wings are shaded with black pigment and all the longitudinal veins are cloudy, showing much darker shading than the rest of the wing. The pupal case has an area, about one-eighth of an inch on the anterior end, which is darkened to black. Mutant expressed in heterozygote.

19. *extended-1;* 2007; autosomal recessive. One or both wings are spread. Expression varies.

20. *extended-2;* 2015; autosomal recessive. One or both wings are spread. Viability and fertility are reduced.

21. *extreme;* 2007; autosomal recessive. All the hairs of the body are reduced to a minute size and some are missing. Males sterile.

22. *fan;* 2020; autosomal recessive. The wings are fan-shaped with cut ends. There is heavy dark hair growth along the distal edges. Similar to *dumpy* (2015).

23. *fused;* 2020; autosomal recessive. In the females the eyes contain large blister-like facets; the pile may be absent or abnormal in arrangement. The abdomen shows

abnormal tergite formation and some absence of pigment. The males do not show as an extreme expression.

24. *gapped;* 2015; autosomal recessive. The second longitudinal wing vein is shortened from one-half to one-fourth the normal length.

25. *grooveless;* 2015, 2017; autosomal recessive. The line of demarcation between the scutellum and thorax is obliterated by tissue growth.

26. *haltere;* 2015; probably autosomal recessive. The halteres are curved downward under the abdomen.

27. *hooked;* 2015; autosomal recessive. The bristles are reduced in diameter and length. The ends of the bristles are blunt in young flies and sometimes branched near the ends. Aged flies have only short blunt bristles since the ends are easily broken off.

28. *irregular;* 2020; autosomal recessive. The hairs and bristles are irregular in the direction of growth. One or both wings may be spread.

29. *lustrous;* 2018; autosomal recessive. The eye is bright red and darkens somewhat in color when aged.

30. *mahogany* (1–4); 2007, 2015, 2018; autosomal recessive. The eye color is a deep, dark red. No allele test completed.

31. *missing-like;* 2007; autosomal recessive. In the females all of the long bristles of the head and thorax are absent. The wings are abnormal in texture and curved over the end of the abdomen. The eyes are rough in texture. The males are less extreme. Males and females are reduced in viability and completely sterile.

32. *mottled;* 2007; autosomal recessive. The eye color is a translucent, light orange with a rather dense, deep layer of dark pigment specks. Viability and fertility are reduced.

33. *mottled-localized;* 2016; autosomal recessive. An area involving from ten to fifteen facets shows dark pigmentation. This area is localized in the anterior medial part of the eye. Late emerging and sterile.

34. *narrow;* 2015; autosomal recessive. The wings are more narrow than normal and have pointed ends. Other pleiomorphic effects are abnormal facet arrangement, shortening of tarsal joints and wing modifications.

35. *narrow-broken;* 2012; autosomal recessive. The wings are shorter and more narrow than normal. The posterior crossvein may be present, gapped or missing entirely.

36. *plexus;* 2020; autosomal recessive. A plexus of extra wing veins occurs between the second longitudinal wing vein and marginal vein.

37. *pointed* (1–4); 2015, 2020; autosomal recessive. The wings are more narrow and slightly shorter than normal. The posterior crossvein may be broken or missing completely. No allele test completed.

38. *pointed-like;* 2015; autosomal recessive. The wings are short and slant to a point on the ends. The center portion is wide and the ends curve downward over the end of the abdomen.

39. *ragged;* (1–7); 2007, 2012, 2014, 2015; type of inheritance not determined. Irregular notched places from the wing occur around the entire edge. No affected females have been recovered and the males show about 50% expression. No allele tests.

40. *rough* (1–11); 2007, 2013, 2014, 2015, 2020, 2021; autosomal recessive. This classification includes a large number of morphologically rough eyed mutants. When allele tests have been completed specific names will be assigned to the different mutants.

41. *roughened;* 2015; autosomal recessive. The facets are abnormal in arrangement and occasionally swollen. Abnormal pile arrangement coincides with facet anomalies. This mutant is nonallelic to *roughoid-2* (2015) and *roughest* (2007).

42. *rougher;* 2015; autosomal recessive. The facets of the eye are abnormal in arrangement with an irregular pattern of blister-like facets. The wings are small in size with large wing cells. Small notched places in the wing sometimes occur.

43. *roughest;* 2007; autosomal recessive. The facets are swollen, blister-like and abnormal in arrangement. Non-allelic to *roughened* (2015) and *roughoid-2* (2015).

44. *roughoid-1;* 2015; autosomal recessive. The eye facets have a slight abnormal arrangement and the pile is missing in spots.

45. *roughoid-2;* 2015; autosomal recessive. The eye facets are abnormally arranged with a fusion of several facets and blister-like facets occurring only rarely. Non-allelic to *roughened* (2015) and *roughest* (2007).

46. *rough-absent;* 2012; autosomal recessive. The eye facets are abnormally arranged and certain bristles of the head and thorax are missing. One or both sexes sterile.

47. *rough-broad;* 2020; autosomal recessive. The eye facets are abnormal in arrangement and blister-like. The wings are usually short, broad and very rounded on the distal end. Small notched places occur around the edge of the wing and extra wing veins extend from the second longitudinal vein to the marginal vein. Shortening of the leg joints and abnormal leg development occur occasionally. Viability and fertility are reduced.

48. *rough-cut;* 2015; autosomal recessive. In the males the entire surface of the eye is covered with swollen, abnormal facets. The wings are sometimes slightly spread and large notched places occur along the edge of the wing. The females are less extreme.

49. *rough-extreme;* 2007; autosomal recessive. Mosaic patches of irregular facets which have dark pigmentation are scattered over the entire eye. The wings are rough in texture and contain a plexus of extra wing veins from the posterior crossvein and longitudinal veins. Certain bristles of the head and thorax are absent.

50. *rough-grooveless;* 2015.1h; autosomal recessive. The facets are misplaced and/or swollen and may show dark pigmentation. The wings and halteres are bent downward, parallel to the body. The line of demarcation between the thorax and scutellum is obliterated.

51. *rough-missing;* 2019; autosomal recessive. The eye facets are irregular and the wings are shorter and more narrow than normal. The wing cells are larger in size than normal. A few or nearly all the long bristles of the thorax may be missing. Viability is low.

52. *rough-mottled;* 2020.1a; autosomal recessive. The facets which may be swollen, blistered, fused and darkly pigmented give a mottled appearance to the eye. The ocellar, humeral, dorso-central, postvertical and/or sternopleural bristles may be missing.

53. *rough-nicked;* 2021.1a; autosomal recessive. The eye facets are irregular and swollen. Small nicked places occur along the edge of the wing.

54. *rough-short;* 2020; autosomal recessive. The facets are irregular and the wings are narrow and short. The abdomen is somewhat short in proportion to the thorax and sometimes shows an absence of pigment and hairs. The orbital, scutellar and ocellar bristles may be missing.

55. *rough-vestigial;* 2012.1c; autosomal recessive. The eye facets are swollen and blister-like. The wing is fan-shaped and heavy hair growth occurs along the distal edge. The expression is variable and extreme forms are sterile.

56. *scarlet-like;* 2007; autosomal recessive. The eye is translucent and bright orange upon emergence but darkens to almost normal in two or three days. Suppressors are probably present in some strains.

57. *scutellar-like;* 2015; type of inheritance not determined. Certain bristles of the thorax are missing but the basal discs are not removed. The absence of the posterior scutellars is the most constant expression. About 75% expression at 25 degrees Centigrade.

58. *semiplexus;* 2021; autosomal recessive. Extra wing vein fragments extend from the 2nd and 4th veins and/or posterior crossvein.

59. *short;* 2021; autosomal recessive. All the tarsal segments, except the first, are shortened and a swollen mass is present on the distal end of each affected segment. The posterior crossvein is sometimes gapped but is never missing entirely. The wings may be narrow or wide and rounded.

60. *short 4th;* 2015.1b; autosomal recessive. The fourth longitudinal vein is shortened.

61. *short 5th-1,* -2; 2007; autosomal recessive. The longitudinal five wing vein is shortened. The expression varies and some strains overlap normal.

62. *short 5th*-3, -4; 2014; autosomal recessive. The 5th longitudinal wing vein is shortened to about two-thirds the normal length. The posterior crossvein is sometimes missing.

63. *short 5th* (5–7); 2015; 2020; autosomal recessive. The 5th longitudinal wing vein is shortened.

64. *short 5th-8;* 2021; autosomal recessive. The fifth longitudinal vein is short. Non-allelic to *short veins* (2015).

65. *shortened;* 2021; autosomal recessive. The wings are more narrow and shorter than normal. Complete separation from normal is difficult.

66. *short veins* (a–c); 2015; autosomal recessive. All the longitudinal wing veins are shortened with the fifth vein being the most extreme in expression and most often affected. In some strains certain bristles of the head, especially the ocellar, and of the thorax are missing. All three mutants are allelic. They give a slight expression with *veinlet* (2007.1d). Non-allelic to *short-5th* (2021).

67. *short veins;* 2017; autosomal recessive. The longitudinal veins, especially the second and fifth, are shortened.

68. *small;* 2012; autosomal recessive. The bristles are reduced in diameter.

69. *small-absent-1* -2; 2015; autosomal recessive. The bristles and hairs of the body are reduced in length and diameter. Certain bristles of the head and thorax are missing. The abdomen sometimes shows abnormal sternite and tergite arrangement and the absence of pigment. Late emergence with low viability and fertility.

70. *small bristles-a,* -b; 2007; autosomal recessive. The bristles are reduced in length and diameter.

71. *small bristles* (1–8); 2015, 2020; autosomal recessive. These eight mutants show a reduction in the bristle size. There is a reduction in the diameter in all cases and in the length except in one case. Other than a quantitative difference, minor qualitative differences as the absence of bristles also occur. In most cases either the male, female or both are sterile.

72. *small extra;* 2015; autosomal recessive. The wings are small in size but the wing cells are much larger than normal. The wing veins are wider than normal and extra veins branch from the fourth longitudinal vein. The abdomen is short with abnormal sternite and tergite formation. The microchaetae of the abdomen are irregular in arrangement.

73. *small wing-1;* 2015; autosomal recessive. The wings are normal in shape but are reduced in size and shortened in length.

74. *small wing-2;* 2018; autosomal recessive. The wings are short and dusky in color.

75. *spread-1;* 2015; autosomal recessive. The wings are spread in a horizontal plane or folded downward and curved under the thorax. Developmental modifications as duplicated portions of the dorsal side of the thorax and shortened tarsal joints also occur. Fertility is low.

76. *spread-2;* 2015; autosomal recessive. Both wings are spread from 45–90 degrees in a horizontal plane or folded under the thorax.

77. *spread-semilethal;* 2020; type of inheritance not determined. One or both wings may be spread. The wings have a pebbly appearance with irregular vesiculate spots occurring at times. The bristles may or may not be reduced in size. Only affected males were recovered.

78. *stocky-1,* -2; 2020; autosomal recessive. The femur and tibia are shortened, enlarged and knotty. Some strains show an absence of bristles and abnormal mounds of tissue on the femur. Late emergence and low viability.

79. *strap;* 2012; autosomal recessive. About two-thirds of the 3rd posterior wing cell, all of the 2nd posterior wing cell and about one-half of the marginal wing cell are

missing. The cut edges are smooth with no hair growth. There is some variation in expression but the mutant usually does not overlap normal. The viability is somewhat reduced.

80. *stubby-1*, -2; 2020; autosomal recessive. The tibia and tarsus joints of the second and third legs are shortened and knotty.

81. *stubby-3;* 2021; autosomal recessive. The second and third legs show abnormal development of the femur, tibia and tarsal segments. Most affected individuals emerge from the pupal case with very abnormal body development and die very young. Not included in Tables.

82. *thickened;* 2007; autosomal recessive. Irregular thickened areas occur along the longitudinal wing veins two, four and five.

83. *translucent;* 2021; autosomal recessive. The eye is translucent and bright red-orange.

84. *veinlet;* 2007; autosomal recessive. The fourth and fifth longitudinal wing veins are shortened. The wing is more narrow than normal and pointed. There is a slight expression with *short veins-b* and *-c* (2015).

85. *wide;* 2013; autosomal recessive. The wings are increased in width across the median part and the wing cells are larger than normal. The entire edge of the wing is usually rolled under to produce a slight arc.

V. INTERSPECIFIC GENE VARIABILITY IN THE VIRILIS SPECIES GROUP

MARY L. ALEXANDER, R. B. LEA, AND W. S. STONE

The *virilis* group has been investigated extensively in the past thirteen years. One of the basic characteristics of this group is its genetic variability. This shows as a high incident of mutations in populations of *Drosophila virilis* Sturtevant, as reported early by Metz, Moses anl Mason (1923), and by Chino (1937, 1941). The species *Drosophila americana americana* Spencer, *Drosophila americana texana* Patterson, Stone and Griffen and *Drosophila novamexicana* Patterson also show this type of mutational variability (Alexander, 1952).

It is difficult to prove that these mutational differences are in fact a basic material for evolution but there is no doubt that genetic isolation factors that reduce or prevent crossing between species are of great evolutionary importance. Genetic differences between strains which determine the type and amount of isolation between species are very common in this group. The earliest tests which showed these differences were those carried out by Spencer (1938, 1940 a, b) using strains of *virilis* and *americana*. Stalker (1942) extended these observations using several strains of the latter species. These species showed decided differences in the number of offspring produced in crosses between different strains and in the amount of sexual isolation between them.

Patterson, Stone and Griffen (1940, 1942) made a systematic study of strain differences in isolating factors in *virilis, americana* and *texana*. In addition to variations in sexual isolation, there were differences in the very effective gamete mortality system of isolation. Sperm from alien males present in the receptacles of inseminated females were motile and able to effect fertilization for a much more limited period of time than were those in homogamic matings. These authors also found a complementary gene action system affecting fertility among F_2 recombination males. In males with an *americana* or *texana* Y chromosome, the 2–3 fusion and the 5 chromosome from this species were necessary at least heterozygous for male fertility anl neither could be replaced successfully by the corresponding *virilis* chromosome.

Patterson and Griffen (1944a, b) gave evidence that a similar variability in the genetic mechanisms determining isolating existed in the montana complex of the *virilis* species group. Patterson (this bulletin) presents evidence on this variable complex as well as the available data on *Drosophila littoralis* Meigan. Patterson, McDanald and Stone (1947) gave added data on the isolating mechanisms in this group while Patterson and Stone (1949) gave details of crosses with a few strains of *novamexicana*. Patterson and Stone (1952) have reviewed the information on these types of variability.

This paper presents further information on *novamexicana* and the western *americana* as well as added information of the Y-autosome balance systems of some of these species.

EXPERIMENTAL

The *texana* and *americana* Y-autosome complementary fertility factors show that the Y is dependent on the presence of two autosomes for fertility. It was desired to test males carrying the *virilis* Y to determine if specific autosomes were necessary for male fertility in this case. The absence of suitable known multiple mutant marker stocks in *americana, texana* and *novamexicana* made it necessary to use the *virilis* marker genes to make this test. An example of the crosses necessary for this test are as follows:

P_1 *novamexicana* ♀ × *b* (2), *tb gp* (3), *cd* (4), *pe* (5) *virilis* ♂
F_1 ♂ × *novamexicana* ♀
F_2 ♂ individually × *b, tb gp, cd, pe* ♀

The genotype of the fertile F_2 males can be determined in the F_3. In case *texana* is used, the 2–3 fusion reduces the recombination classes by half, while if *americana* is used, all males receive the *virilis* 4 since the americana X-4 fusion causes the free 4 chromosome to separate from it with the Y.

One hundred F_2 males were tested for each of the three wild species, Table 1. Despite the small number of fertile males, the results are con-

TABLE 1

The *virilis* y-autosome Fertility Relations

Wild species used	*virilis* autosomes present in fertile males							
(see text for crosses)	2 (b)	2	2	2				
	3 (tb) (gp)	3			3			
	4 (cd)		4			4		
	5 (pe)	5	5	5	5	5	5	
(1) *novamexicana* (1714.4)	3	3	5	2	1	1	2	
(2) *texana* (1128.10)	12	3				5	2	
(3) *americana* (1760.8i)	10					12		

sistent in showing that the *virilis* 5 chromosome must accompany the *virilis* Y if males are to be fertile. No other chromosome proved necessary for fertility.

Western *americana* strains have not been found in association with *novamexicana*. In fact there is a gap of several hundred miles between the *novamexicana* strains found in the Rocky Mountains and the western *americana* strains collected in *Nebraska*. There is cytologically more resemblance between these forms than between *novamexicana* and eastern *americana* or *texana*. In addition there exists in populations of *americana* from Chadron, Nebraska, two color phases; the regular dark type and a light type resembling *novamexicana*. Athough homozygous light indivi-

duals have not been collected, both color phases have been recovered from collections on several occasions at this locality and at other localities (Alexander, 1952). Table 2 presents the P_1 and F_1 crosses between *nova-*

TABLE 2

Test of *novamexicana* (1714.4) and western *americana* (1773.43e)

Cross ♀ ♂	Number tested	Percent fertile	Average per vial (5 days)	Sex ratio dark ♀	light ♀	dark ♂	light ♂
$A_D \times A_L$	71	42	29	247	315	283	314
$A_L \times A_D$	90	17	29	115	144	111	153
$A_D \times N$	96	58	34	275	412	195	378
$A_L \times N$	122	34	33	0	1094	0	929
$N \times A_D$	36	58	....	.....	.....	.....	.....
$N \times A_L$	41	54	....	.....	.....	.....	.....
$(A_DN)_D \times (A_DN)_D$	63	92	37	637	920	856	889
$(A_DN)_L \times (A_DN)_L$	66	85	56	0	2846	0	2880
$(A_DN)_D \times N$	109	90	35	577	1138	575	1030
$(A_DN)_L \times N$	73	69	45	0	2646	0	2249
$N \times (A_DN)_D$	111	89	15	249	440	333	353
$N \times (A_DN)_L$	109	73	11	0	647	0	634

mexicana and the two color phases from a single stock of *americana* isolated at Chadron. The light color phase is recessive but the ratios obtained were not too satisfactory. The color factors are not sex linked. Crosses of light females by light males do not produce dark offspring, but a modifying factor does cause some of them to darken. As these are not dark, the data suggests a major pair of factors with dark dominant but less viable than light in crosses. Crosses given in Table 2 show that the light factor is the same as that in *novamexicana* for all progeny are light.

The *americana* stock is not very fertile in crosses of light by dark, even though the dark used was heterozygous for light for the most part. In fact both the P_1 and F_1 crosses of dark and light went better with *novamexicana* than with each other. There seems no very effective P_1 or F_1 isolation between these strains of the two species. This situation is very different from that found by Patterson and Stone (1949) in equivalent crosses between *novamexicana,* from San Antonio, New Mexico, and eastern *americana,* the Anderson, Indiana, strain. In those earlier crosses 15% of the pairs were fertile to produce an average of 28 flies per pair if *americana* was the female parent but less than one percent of the reciprocal crosses produced offspring. The $F_1 \times F_1$ were only 2% fertile in either case although the number of F_2 progeny per pair were equivalent to those in Table 2. Back crosses of the F_1 ranged in fertility from less than one percent (*novamexicana* ♀ × *americana/novamexicana* hybrid ♂) to 19 percent with progeny averages around 28 per pair. The Chadron, Nebraska stock is quite different from the Anderson, Indiana stock in fertility of the P_1 crosses with *novamexicana* and in the F_1 fertility.

A series of tests were run to investigate the differences in cross fertility and F_1 hybrid fertility between three stocks of *virilis* on one hand and two different stocks each of *novamexicana*, western *americana* and eastern *americana*. The original tests by Patterson, Stone and Griffen (1940, 1942) indicated that the Henly, Texas (86.4) stock of *virilis* was much more isolated from the wild species than any other *virilis* strain, including that from Pasadena. The two new strains of *virilis* tested are much more crossfertile with some of the other species than is Pasadena. The percentage of fertility for the crosses given in Tables 3 and 4 are based on 100 pairs tested.

Table 3 shows that the Pasadena *virilis* is much more isolated from both strains of *novamexicana* than is either the Mexican or Argentinian strain. This difference is due to the greater number of fertile pairs when *virilis* is

TABLE 3

Percent of pairs fertile in P_1 crosses

americana or *novamexicana* strains and origin	*virilis* strains and origin: Pasadena, California ♀	Pasadena, California ♂	Texmelucan, Puebla, Mexico 1801.1 ♀	Texmelucan, Puebla, Mexico 1801.1 ♂	Chaco, Argentina 1999.1 ♀	Chaco, Argentina 1999.1 ♂
novamexicana						
1714.4 (San Antonio, New Mexico)	24	25	69	10	95	36
1954.3a (Whitewater, Colorado)	34	3	72	14	92	19
americana western						
1761.9s (Chinook, Montana)	23	52	60	41	41	35
1773.4e light (Chadron, Nebraska)	48	24	35	40	30	39
1773.4e dark (Chadron, Nebraska)	61	51	33	38	16	27
americana eastern						
Anderson (Anderson, Indiana)	29	19	12	19	20	17
1882.6 (Millersburg, Penn.)	34	21	6	45	38	55

TABLE 4

Percent of Fertile F_1 tests

P_1 crosses	$F_1 \times F_1$	F_1♀ × V♂	F_1♀ × N or A ♂	F_1♂ × V♀	F_1♂ × N or A ♀
1714.4♀ × 1999♂	100	100	98	91	100
1714.4♀ × 1801.1♂	77	100	100	100	65
1999♀ × 1714.4♂	0	100	97	0	0
1801.1♀ × 1714.4♂	0	90	78	0	0
Pasadena ♀ × 1714.4♂	0	97	43	0	0
1999♀ × 1954♂	7	100	100	13	0
1801.1♀ × 1954♂	8	100	52	3	0
Pasadena ♀ × 1954♂	1	100	100	3	1
1999♀ × 1761.9s♂	100	96	92	74	93
1801.1♀ × 1761s♂	100	100	50	100	90
Pas ♀ × 1761.9s♂	83	92	100	77	89
1761.9s♀ × 1999♂	100	100	100	100	90
1761.9s♀ × 1801.1♂	81	100	100	79	65
1761.9s♀ × Pas ♂	81	100	100	61	50
Pas ♀ × Anderson ♂	100	100	59	53	31
Pas ♀ × 1882 ♂	100	99	82	100	62
1882♀ × 1999♂	100	91	61	100	98
1882♀ × 1801.1♂	71	97	100	98	82
1882♀ × Pas ♂	38	82	53	66	80
Anderson ♀ × Pas ♂	100	100	76	100	76
Anderson ♀ × 1999 ♂	88	73	56	97	20
Anderson ♀ × 1801.1♂	86	100	60	96	58

the female parent. The three *virilis* strains do not show the same differential in isloation to the strains of *americana*. In fact there exists the usual unpredictable pattern of variation in number of crossfertile pairs. There is no doubt that genetic differences exist between all these strains which are reflected in the variations in isolation that exists between them.

The F_1 tests show some fundamental differences in the fertility of these hybrid combinations. First, certain differences in the fecundity of the P_1 crosses, measured as number of viable F_1 available to test, have prevented tests of some F_1 combinations. We have no quantitative measure of the difference but can only point out that certain P_1 crosses, though fertile, produced so few offspring that F_1 tests were impracticable. In fact a number of mass matings were made of the P_1 crosses in addition to the pairs listed in Table 3, yet despite repeated crosses between the combinations that gave a poor yield, certain F_1 classes were too few to test. As a certain percentage of all crosses are somewhat fertile, this is probably due to differences in effective survival and utilization of alien sperm such as that demonstrated previously by Patterson, Stone and Griffen (1942).

The females of the San Antonio, New Mexico, strain of *novamexicana* did not produce many offspring when crossed to Pasadena *virilis* males. Patterson and Stone (1949) reported that the $F_1 \times F_1$ pairs were only two percent fertile. The hybrid males were very infrequently fertile in back crosses, from one to eight percent. The hybrid females were 71% fertile to Pasadena *virilis* but only 18% fertile to *novamexicana* males. In the reciprocal cross these earlier tests gave a lower fertility of these hybrid females backcrossed to Pasadena *virilis* males but the cross to *novamexicana* males gave identical results with that listed in Table 4. No fertile matings were recorded in Table 4 for the *virilis-novamexicana* (1714.4) hybrid males but Patterson and Stone (1949) obtained one or two percent fertility.

In contrast to the lesser fecundity when Pasadena *virilis* was used as a male parent, the Mexican and Argentinian strains produced a number of offspring with the New Mexican *novamexicana* females. These were quite fertile on inbreeding and backcrossing, Table 4. In the reciprocal crosses, using *virilis* strain females, the F_1 males were sterile while the females were quite fertile.

The other *novamexicana* stock from Whitewater, Colorado, showed several differences in behavior. The females did not produce sufficient offspring with the three strains of *virilis* to give satisfactory F_1 tests. In reciprocal crosses some F_1 males from all three combinations were fertile, and the only differential shown in the test of the females was the low fertility of one combination, Table 4.

In the Chadron, Nebraska stock of western *americana*, both light and dark strains failed to produce enough offspring for the several tests in reciprocal crosses to any *virilis* strains while the other stock from Chinook, Montana produced a satisfactory number of hybrids. These were quite fertile in most combinations.

Eastern *americana* strains were less fertile with the *virilis* strains in some of the P_1 crosses. Certain tests showed an exceptional reduction in fertility. Most of the tests of the F_1 hybrids from a cross of the Millersburg strain of *americana* by Pasadena *virilis* males were less fertile than the hybrids from other equivalent tests. The $F_1 \times F_1$ fertility was especially low. The backcross to *americana* females of the F_1 males from Anderson *americana* by the Argentinian *virilis* strain went only slightly. The other crosses in Table 3 failed to produce enough hybrids for F_1 tests in these experiments.

Certain additional crosses give information on other sources of isolation. Table 5 presents data on the crosses and backcrosses using the Mexican

TABLE 5

Crosses between *virilis* (1801.1) and *novamexicana* (1714.4)

Cross	Number Tested	Percent Fertile	Average per vial (5 days)	Sex-Ratio ♀ ♂
V × N	102	60	16	1032–1037
N × V	90	2	2	
NV × NV	103	94	28	1336–1131
VN × VN	108	7	1	4–3
V × VN	108	33	7	94–111
N × VN	99	7	1	
VN × V	122	98	47	758–709
VN × N	106	62	37	1498–1404
(VN) V × (VN) V	91	75	24	955–797
(VN) N × (VN) N	117	55	21	1023–911
V × (VN) V	104	90	54	2351–2263
N × (VN) V	109	26	5	106–76
(VN) V × V	152	86	43	3124–2395
(VN) V × N	109	46	21	927–705
V × (VN) N	78	56	49	972–919
N × (VN) N	58	45	39	731–694
(VN) N × V	82	58	11	417–418
(VN) N × N	71	37	23	499–461

strain of *virilis* and the New Mexican strain of *novamexicana*. In a test to determine the cross fertility of this strain of *virilis* females when crossed with *novamexicana* males from Whitewater, Colorado, 94 of 100 pairs were fertile. They produced an average of 16 flies per pair over a five day test period, yielding 412 females and 448 males. The cross was, therefore, somewhat more successful. In the test given in Table 5, the sex ratios depart from the expected 1:1 ratio in some of the crosses. It is not clear whether certain gene combinations among the males are less viable or if the hybrid males are simply less viable than the corresponding hybrid females. It is apparent that where crossing over can increase the recombinations between the alien genotypes, the viability of hybrid males is reduced further as a consequence. The F_1 *virilis/novamexicana* hybrid males are not very fertile but the F_1 females from both reciprocal matings are quite often fertile, as are the *novamexicana/virilis* hybrid males. Tests of the F_2 hybrids indicate that crosses back to the recurrent parent are more often fertile, and that hybrids from crosses back to *virilis* are more often normal

in fertility. It seems that the *virilis* genotype can tolerate a heterozygous dilution with *novamexicana* genes and still be fertile much more effectively than the *novamexicana* genotype can tolerate the *virilis* genes even heterozygous.

An egg hatch was run with two combinations shown in Table 5, the inbred test of *novamexicana/virilis* hybrid females × males, and *virilis/novamexicana* females back to virilis males. In the former 672 out of 1738 eggs hatched or 38.7 percent, while in the latter only 624 out of 2054 or 30.4 percent were viable combinations. These data suggest that *virilis/novamexicana* gene recombinations are more often viable when *novamexicana* genes are homozygous.

DISCUSSION

The genetic systems of the species studied have diverged in a number of respects that lead to one or another type of isolation. A good demonstration of this is found in the gene systems that determine male fertility. This was tested by substituting alien autosomes homozygous with a Y chromosome from a particular species. In tests between *americana, texana* and *virilis,* the F_1 hybrid males are fertile. Those produced by crossing *virilis* by *novamexicana* are fertile if the latter species is used as the female parent, and some are fertile when the Colorado stock of *novamexicana* is used as the male parent. Patterson, Stone and Griffen (1940) recorded evidence that the fertility of male hybrids between *virilis* females and *texana* or *americana* males depended on factors in the Y, the 2–3 fusion and the 5 chromosome for if these F_1 males were backcrossed to *virilis,* all this complex of chromosomes is necessary for F_2 male fertility. The *virilis* male fertility gene complex involves only the Y and 5 chromosomes for all other autosomes can be replaced homozygous with their equivalents from *americana, texana* or *novamexicana,* Table 1.

Patterson and Stone (1949) demonstrated that gene recombinations between *novamexicana* and *texana* or *americana* were often inviable. There was a negative correlation between the amount of gene recombination and viability since the F_2 progeny from backcrosses of F_1 hybrid females were less viable than those from F_1 hybrid males. This paper presents further evidence of recombination infertility, Table 5. Egg-hatch counts from hybrid combinations indicate that recombinations of *virilis* and *novamexicana* genes are more often viable if the *novamexicana* genes are homozygous.

Variation in genotype associated with variation in cross fertility of different strains of one species with different strains of another has been demonstrated by many authors. Tables 3, 4, and 5 give still further examples. The absence of F_1 tests in Table 4 despite some fertility between the P_1 strains given in Table 3 is a rough measure of differential cross fecundity as measured by the number of F_1 hybrids produced from the several P_1 crosses. These tables show the typical differences in effectiveness of species crosses as determined by differences between strains.

The two color phases of the western *americana* from Chadron, Nebraska, and the lack of serious genetic isolation between this strain of *americana* and the New Mexican strain of *novamexicana* are in agreement with the greater cytological similarity of these forms (Hsu, 1952). The fact that the light phase is genetically apparently equivalent to the color system of *novamexicana* suggests that these species are more closely related genetically in their region of distribution where they are even now fairly close together geographically, Table 2.

There has been found no zone of contact between these species so the genetic similarity must represent a residual genetic similarity from past hybridizations. This is a case where different strains of *americana* show very different amounts of isolation factor divergence with another species, *novamexicana,* for Patterson and Stone (1949) have shown that eastern *americana* and *novamexicana* are genetically quite isolated and consist of different balanced gene systems.

BIBLIOGRAPHY

Alexander, Mary Louise, 1952. Gene Variability in the *americana-texana-novamexicana* complex of the virilis group of Drosophila. This bulletin.

Chino, M., 1937. The Genetics of *Drosophila virilis.* Jap. J. Genetics, 12: 189–210, 257–277; 13: 100–120.

Chino, M., 1941. New Mutants in *Drosophila virilis.* Jap. J. Genetics, 17: 185–206.

Hsu, T. C., 1952. Chromosonal variation and Evolution in the virilis group of Drosophila. This bulletin.

Metz, C. W., Moses, L. S., and Mason, E. D., 1923. Genetic studies on *Drosophila virilis* with considerations on the genetics of other species of Drosophila. Publ. Carn. Ins., 328: 94 pp.

Patterson, J. T., and Griffen, A. B., 1944a. Relationships of *Drosophila montana* and *D. lacicola* to other Members of the Virilis Group. Univ. of Texas Publ., 4445: 194–211.

Patterson, J. T., and Griffen, A. B., 1944b. A Genetic Mechanism Underlying Species Isolation. Univ. of Texas Publ., 4445: 212–223.

Patterson, J. T., McDanald, L. W., and Stone, W. S., 1947. Sexual Isolation Between Members of the Virilis Group of Species. Univ. of Texas Publ., 4720: 7–31.

Patterson, J. T., and Stone, W. S., 1949. The Relationship of *novamexicana* to the other Members of the Virilis Group. Univ. of Texas Publ., 4920: 7–17.

Patterson, J. T., and Stone, W. S., 1952. Evolution in the Genus Drosophila. In press.

Patterson, J. T., Stone, W. S., and Griffen, A. B., 1940. Evolution of the Virilis Group in Drosophila. Univ. of Texas Publ., 4032: 218–250.

Patterson, J. T., Stone, W. S., and Griffen, A. B., 1942. Genetic and Cytological Analysis of the Virilis Species Group. Univ. of Texas Publ., 4228: 162–200.

Spencer, Warren P., 1938. *Drosophila virilis americana,* a new subspecies. Genetics, 23: 169–170.

Spencer, Warren P., 1940a. Subspecies, hybrids and speciation in *Drosophila hydei* and *Drosophila virilis.* Amer. Nat. 74: 157–179.

Spencer, Warren P., 1940b. Levels of divergence in *Drosophila* speciation. Amer. Nat. 74: 299–311.

Stalker, H. D., 1942. Sexual isolation studies in the species complex *Drosophila virilis.* Genetics, 27: 238–257.

VI. A PAIR OF ALLOPATRIC SUBSPECIES BELONGING TO THE REPLETA SPECIES GROUP

J. T. PATTERSON

INTRODUCTION

Several years ago we described a new member of the repleta group from Mexico under the name *Drosophila fulvimacula* (Patterson and Mainland, 1944). Up to that time it had been collected in four different states, as follows: San Luis Potosí, at Huichihuayan; Hidalgo, at Ixmiquilpan; Veracruz, at Puente Nacional and Jalapa; and Guerrero, at Taxco. It was later taken (Sept. 6, 1947) by M. R. Wheeler and F. A. Cowan in the state of Oaxaca at a point located about sixty miles south of the city of Oaxaca, and from this collection a laboratory stock (No. 1808.37) was established. This has enabled us to determine several facts of interest not included in the original description. Thus, Ward (1949) showed that the metaphase plate has five pairs of rods and a pair of dots, with the longest pair of rods representing the sex chromosomes. He was also able to determine that the salivary gland nuclei contain five long strands and a dot.

In November 1949 the writer received from Professor Th. Dobzhansky of Columbia University a culture of flies which resembled very closely those of *D. fulvimacula*. A comparative study of the two forms lead to the conclusion that they represented a pair of subspecies. The Brazilian form is described below, under a subspecific name first suggested by Dr. C. Pavan, as *Drosophila fulvimacula flavorepleta*, while the Mexican form becomes *D. fulvimacula fulvimacula*.

Drosophila fulvimacula flavorepleta. subsp. nov. Patterson and Pavan.

On account of the close resemblance of the two subspecies, it does not seem necessary to give a detailed description of the Brazilian form. Dr. C. L. Ward has studied the cytology of this subspecies and reports that its

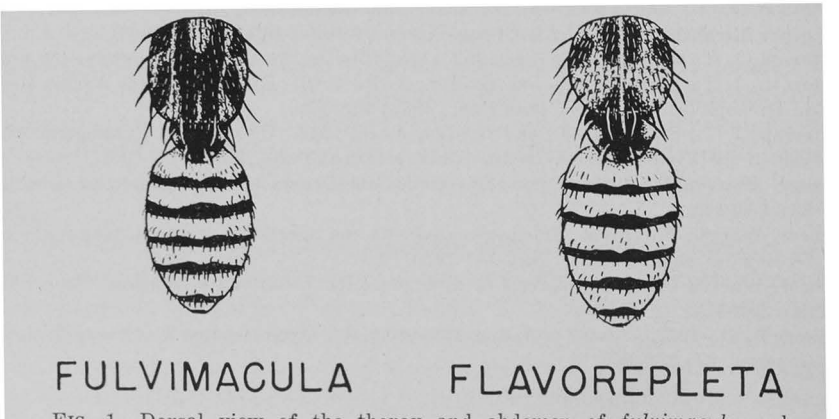


FIG. 1. Dorsal view of the thorax and abdomen of *fulvimacula* and *flavorepleta*, showing the differences of the mesonotal color patterns of the two subspecies.

metaphase plate is identical with that of *fulvimacula*, and that the salivary gland chromosomes of the *fulvimacula/flavorepleta* hybrid larvae were completely synapsed. The best diagnostic character difference between the two forms is the color patterns of the mesonotum, which is darker in *fulvimacula*. Moreover, in *flavorepleta* there is a fine stripe at mid-line which extends from the anterior to the posterior margins of the mesonotum, and sometimes onto the scutellum (Fig. 1). This character difference enables one to separate these two subspecies.

Types.—Holotype male, 4 paratype males and 5 paratype females (No. 1980.1) from a stock originally collected at Belem, Brazil, deposited in the University of Texas collection.

CROSSES BETWEEN FULVIMACULA AND FLAVOREPLETA

In Table 1 are listed the results obtained from several test crosses that were made in small mass matings of ten pairs per culture (crosses 1–6). In the control tests the homogamic matings of the Mexican form gave 210 offspring with an average of 3.5 per tested female, while those of the Brazilian form yielded 539 with an average of 10.8 per female. Since 60 pairs were used in the first cross and only 50 in the second, it is clear that *flavorepleta* produced about three times as many progeny per female as did *fulvimacula*. In the P_1 matings the M × B cross averaged 9.3 offspring per female, while the B × M cross gave a somewhat lower average of 7.4. The two inbred crosses of MB × MB and BM × BM gave averages of 4.5 and 4.0, respectively. In none of these six crosses is there a significant deviation from a 1:1 sex-ratio.

TABLE 1

Crosses between the subspecies *fulvimacula* (M) and *flavorepleta* (B)

Type		Crosses ♀ ♂	Number tested	Percent fertile	Number of offspring	Females	Males	Average per female
Controls	1.	M × M	60	.....	210	102	108	3.5
mass	2.	B × B	50	.....	539	282	257	10.8
P_1-mass	3.	M × B	160	.....	1499	794	705	9.3
	4.	B × M	280	.....	2090	1035	1055	7.4
Inbred	5.	MB × MB	90	.....	405	211	194	4.5
mass	6.	BM × BM	150	.....	602	295	307	4.0
F_1-pairs	7.	MB × MB	120	25%	754	378	376	24.3
F_1-pairs	8.	BM × BM	124	33%	1072	570	572	26.1
F_2-pairs	9.	MB × MB	140	8.6%	480	247	233	40.0
F_2-pairs	10.	BM × BM	140	5.7%	296	168	128	37.0

Inbred tests by the use of pair matings were carried out for the F_1 and F_2 generation flies (Table 1, 7–10). In the F_1 cross of MB × MB, 25% of the cultures produced offspring, yielding 754 flies for an average of 24.3 per vial. In the BM × BM cross 33% of the cultures were fertile and produced 1072 flies for an average of 26.1 per vial. Thirty females from the sterile cultures of the first cross were dissected and their sperm receptacles examined for the presence or absence of sperm. Twenty of these showed no sperm present, while nine had motile and one non-motile sperm

in their receptacles. Thirty females from the sterile cultures of the second cross were also dissected and examined for sperm. These showed eleven without sperm, while twelve had motile and seven non-motile sperm in their receptacles. Ten males from each of these crosses were dissected, and it was found that all twenty had motile sperm in their gonads. The flies from the remaining sterile cultures were transferred to fresh food and kept for about ten days, but none of them produced offspring. The results from neither of these two crosses shows any significant deviation from a 1:1 sex-ratio.

The results obtained in the inbred tests for the F_2 generation flies are listed in Table 1, crosses 9 and 10. For each of the two crosses 140 pairs were tested for fertility and fecundity. The MB × MB cross gave 8.6% fertile, yielding 480 flies for an average of 40 offspring per female, while the BM × BM cross gave 5.7% fertile, producing 296 flies for an average of 37 offspring per female. For the first cross, the males and females from forty apparently sterile cultures were dissected and examined. All forty males had motile sperm in their testes, but only six females showed sperm in their receptacles, all motile. In fifty-nine of the remaining cultures the males and females were still alive, and these were transferred to fresh food vials. Eight of these laid eggs, and five eventually produced offspring. These five were included in the numbers used to determine the percent fertile.

The males and females from the sterile cultures of the BM × BM cross were also dissected. Thirty-eight of the males had motile sperm in their gonads, and two had non-motile sperm. For the females, it was found that eleven had motile, one had non-motile, and twenty-eight had no sperm in their receptacles. This left sixty-five sterile cultures with both flies still alive and these were transferred to fresh food vials. Two of the females laid eggs, and one eventually produced offspring.

TABLE 2

Backcrosses of F_1 flies to the parental subspecies

Crosses ♀ ♂	Number tested	No. of ♀♀ dissected	Number inseminated	Receptacles mot.	Receptacles non-mot.	Number of offspring	Average per female
1. MB × M	49	9	6	5	1	82	1.6
2. MB × B	60	0	....	....	....	385	6.4
3. BM × M	55	35	20	15	5	90	1.6
4. BM × B	50	0	....	....	....	379	7.9
5. M × MB	58	48	7	1	6	38	0.6
6. B × MB	40	0	....	....	....	463	11.5
7. M × BM	61	11	0	....	....	0	.....
8. B × BM	90	0	....	....	....	630	7.0

One of the more interesting results obtained was from the backcross tests of the F_1 flies to the parental subspecies (Table 2). For these tests mass matings of ten pairs per culture were used. The results fall into two classes: (1) Whenever the F_1 females or males are backcrossed to the Mexican subspecies, the matings go very poorly (crosses 1, 3, 5) or

else not at all (cross 7). In the MB × M cross (1), forty-nine tested pairs gave eighty-two offspring for an average of 1.6 per female, and of the nine dissected females, six had been inseminated. Five of these females contained motile and one non-motile sperm in the receptacles. For the BM × M cross (3), fifty-five tested pairs yielded ninety offspring for an average of 1.6 per female. Of the thirty-five females dissected, the receptacles of twenty showed sperm, fifteen with motile and five with non-motile. The fifty-eight tested pairs of the M × MB cross (5), gave only thirty-eight offspring for an average of 0.6 per female. Forty-eight females were dissected, of which only seven had been inseminated. One of these had motile and six had non-motile sperm in the receptacles. Finally, the M × BM mating (7) proved to be incompatible, and none of the eleven dissected females had been inseminated. (2) Whenever the hybrid males or females were backcrossed to the Brazilian subspecies, all matings went reasonably well (crosses 2, 4, 6, 8) and produced averages of 6.4, 7.9, 11.5 and 7.0, respectively, for the four crosses.

SUMMARY AND CONCLUSIONS

The facts presented above allow for certain conclusions to be drawn. In the first place, the two forms can be separated on the basis of an external character difference—the color pattern of the mesonotum. In the second place, on the basis of the known collection records they occupy different areas of the Western Hemisphere, and in all probability are thus geographically isolated. There is no way of determining how long the two forms have occupied these separate ecological regions, but the time has been sufficient for them to exhibit evolutionary divergence in the form of isolating factors of a genetic nature. These factors are revealed in the results tabulated in Tables 1 and 2.

In Table 1 the average number of offspring recorded per tested female in crosses 1–6 seems rather low, varying from 3.5 to 10.8. But this is due to the fact that small mass matings were used for these tests, and the full number of females were used in calculating these averages. Obviously, not all such females had been inseminated. In contrast to these results, those from the pair-mating tests of the F_1 and F_2 generation flies (crosses 7–10) gave much higher averages, varying from 24.3 to 40. These higher averages are due to the fact that they were calculated on the basis of the number of fertile females only.

The dissections of the females and males from the apparently sterile cultures of the pair-mating tests reveal that two isolating mechanisms are operating to reduce the number of fertile cultures. These results may be summarized as follows: A total of 60 dissected females from the sterile cultures of the two F_1 inbred crosses (7, 8) showed that 29 of them had been inseminated, or about 48.3%; while 80 dissected females from similar cultures of the F_2 inbred crosses (9, 10) revealed that only 18 had been inseminated, or 22.5%. Dissections of 100 males from both types of

crosses showed that 98 contained motile sperm in their gonads, and only two had non-motile sperm. Of the 317 pairs from the remaining sterile cultures of all four inbred crosses that had been transferred to fresh food vials, only six females, or less than 2%, eventually produced offspring.

A brief analysis of these data brings out the following points. Since 98% of the dissected males from the sterile cultures had motile sperm in their testes, it is clear that the low fertility rates cannot be ascribed to a failure of spermatogenesis to produce normal sperm, such as has been reported for some other forms; e.g., in crosses between the subspecies of *D. pallidipennis* (Patterson and Dobzhansky, 1945). In the sterile cultures of the two F_1 inbred crosses less than one-half of the females (48.3%) were found to have been inseminated, and in the two F_2 crosses less than one-fourth had been inseminated (22.5%). This means that sexual isolation is operating to reduce the number of inseminations in these crosses, and that it is more effective in the F_2 than in the F_1 matings.

The dissections have revealed another point of interest in connection with the 317 pairs transferred to fresh food vials. The females from these pairs were not dissected, but only six of them eventually produced offspring. On the basis of the evidence from the dissected females from the same lots, 47 out of 140 were found to have been inseminated (ca, 33%). It is reasonable to assume that about the same percentage of the transferred females would also have been inseminated, and if so, it would indicate the operation of another isolating mechanism, characterized by a failure of the sperm to fertilize the eggs in the receptacles of the females. At the time the 140 females were dissected (15–16 days), in 38 of the 47 inseminated females the sperm were still alive and active (ca, 80%). The reaction may not be of exactly of the same nature as we have reported for several cases for crosses in other forms, in which the sperm soon became inactivated, or even killed, in the reproductive tract of an alien female. Some light has been shed on the probable nature of the reaction in the present cases by a single observation. A female from a pair of flies found in copula was dissected about thirty minutes after the pair had separated. Her reproductive tract gave evidence of a weak type of *insemination reaction*..

The general conclusion is that *fulvimacula* and *flavorepleta* constitute a pair of allopatric subspecies, which have deveoped at least one external character difference and two definite isolating mechanisms.

REFERENCES

Patterson, J. T., and G. B. Mainland. 1944. The Drosophildae of Mexico. Univ. Tex. Publ., 4445:9–101.

Patterson, J. T., and Th. Dobzhansky. 1945. Incipient reproductive isolation between two subspecies of *Drosophila pallidipennis*. Genetics, 30:429–438.

VII. STUDIES IN THE REPLETA GROUP: THE MELANOPALPA SUBGROUP

C. L. WARD AND W. S. STONE

The repleta group of the subgenus Drosophila is interesting for a number of reasons. It is the largest group in the genus, consisting of 53 known forms, including three pairs of subspecies. The large size of the species group is due to the fact that an ancestral form achieved desert adaption so that most of these species live or can live on the desert succulents. This adaptation opened a large area which was invaded and occupied by the rapidly branching evolving group. The group is typically southwestern, but several species or different subspecies occur in South America. We have continued the investigation, begun by Wharton, 1942, 1944, of the members of the melanopalpa subgroup which have produced hybrids.

MATERIALS AND METHODS

The following strains were used in this investigation:

1. *Drosophila canapalpa* Patterson and Mainland. Stock 1402.17 was established from 15 flies which were collected at La Placita near Jacala in the state of Hidalgo, Mexico, in 1943.

2. *Drosophila melanopalpa* Patterson and Wheeler. Stock 1351.7 was made up from 8 specimens collected in 1942 at Laguna Camecauro, Michoacan, Mexico.

3. *Drosophila limensis* Pavan and Patterson. Strain 1529.2a was brought to this laboratory from Lima, Peru, by Dr. C. Pavan.

4. *Drosophila repleta* Wollaston. The following strains were used: 1574.1, Kilgore, Texas; 1797.1, Mexico City, Mexico; 1412.7, Mogi das Cruzes, Sao Paulo, Brazil; and 1529.ya, Corumba, Brazil. Wharton and Sturtevant (1946) studied a strain of *Drosophila neorepleta* from Guatemala. The strain of *melanopalpa* (1244.11) used by Wharton was from Cave Creek, Arizona.

These forms do not cross readily enough to obtain significant results with pair matings. Mass matings using about twenty-five flies of each sex, aged eight to ten days, were made in half pint milk bottles containing the banana-yeast-malt-agar media regularly used in this laboratory. The flies were transferred weekly to fresh food for six weeks and discarded. The cytological observations were made on preparations of larval ganglia stained with aceto-orcein and salivary gland chromosomes similarly stained after prefixation with N HCl. The F_1 or F_2 progeny of each sex from a cross were divided into two equal groups and backcrossed in mass matings.

RESULTS

A summary of the results obtained in these experiments together with the added material from Wharton and Sturtevant is presented in Table 1.

The data for the fertile crosses in our experiments, indicating the number of masses tested, the number of F_1 offspring, both male, female and recognizable intersex, and the fertility of the F_1 in the test indicated are given in Table 2.

TABLE 1

Summary of Results of Fertility Tests in the Melanopalpa Subgroup

♀ ♂	*repleta*	*limensis*	*canapalpa*	*melanopalpa* (Arizona)	*melanopalpa* (Mexico)	*neorepleta*
repleta.....	stock	incompatible	incompatible	incompatible (Wharton)	1 ♀	incompatible (Wharton)
limensis.....	fertile ♀ ♀ sterile ♂ ♂	stock	1 ♀ larval and pupal lethal	not tested	incompatible	not tested
canapalpa	sterile ♀ ♀ sterile ♂ ♂ (Wharton)	2 fertile ♀ ♀ larval and pupal lethal	stock	fully cross fertile (Wharton)	2 ♀ ♀, 2 ♂ ♂	fully cross fertile (Wharton)
melanopalpa.. (Arizona)	sterile ♀ ♀ sterile ♂ ♂ intersexes (Wharton)	not tested	fully cross fertile (Wharton)	stock	not tested	fully cross fertile (Wharton)
melanopalpa.. (Mexico)	fertile ♀ ♀ sterile ♂ ♂ female intersexes (Wharton)	incompatible	1 ♀, 8 ♂	not tested	stock	not tested
neorepleta	fertile ♀ ♀ sterile ♂ ♂ (Sturtevant) sterile ♀ ♀ sterile ♂ ♂ (Wharton)	not tested	fully cross fertile (Wharton)	fully-cross fertile (Wharton)	not tested	stock

In our tests of over thirty mass matings, we obtained no hybrids between *limensis* and *melanopalpa* Mexico. *Drosophila limensis* females, when crossed to *repleta* males produced fertile females and sterile males. It was found upon dissection of these sterile males that the internal genitalia were normal in appearance except for the testes which contained some very large cells, but no spermatozoa. The F_1 females were fertile in back crosses to both parent strains. They produced 13 female and 9 male F_2 in crosses to *repleta* Kilgore; all F_2 proved sterile except a few females which were fertile to *repleta* males. The F_1 females produced 30 female and 34 male F_2 in crosses with *limensis*. The F_2 females were fertile to *repleta* and *limensis* while a few males proved fertile to *repleta*. The few F_3 produced died before they could be tested. The most effective cross in the series was that between *melanopalpa* Mexico females and *repleta* Kilgore males for there were nearly as many F_1 as P_1. The offspring included 588 males, approximately two-thirds of the progeny, 302 females and 42 female-like intersexes, although some of those classed as normal sexes might have been intersexual also. The F_1 were sterile except for a few females that crossed to *repleta* Kilgore to give two sterile F_2 males. The cross was repeated, using the hybrid between *repleta* strains from

Kilgore, Texas, and Corumba, Brazil, as one parent in the hope that it would yield a higher percentage of intersexes, but such was not the case. The female-like intersexes usually showed small claspers, undeveloped ovaries and sometimes abnormal internal genitalia, and males which were dissected showed small, undeveloped testes. Malformed tergites and slightly abnormal genitalia were quite common among the offspring.

TABLE 2

Crosses in the Melanopalpa Subgroup

Cross	Number of Matings (25 ♀ + 25 ♂)	F_1 Progeny ♂	F_1 Progeny intersex	F_1 Progeny ♀	Backcross F_1 ♀ times	Backcross F_1 ♂ times
1. K ♀ × L ♂	34	0		0		
2. L ♀ × K ♂	58	40		36	K + L +	K — L —
3. K ♀ × M ♂	21	0		0		
4. M ♀ × K ♂	19	302	42	588	K + M —	K — M —
5. 1529.ya ♀ × M ♂	8	1		0		
6. M ♀ × 1529.ya ♂	13	1		0		
7. 1797.1 ♀ × M ♂	4	0		0		
8. M ♀ × 1797.1 ♂	7	1		0	M —	
9. M ♀ × 1412.7 ♂	4	1		5		M — 1412.7 —
10. (1412.7 × K) ♀ × M ♂	5	0		0		
11. M ♀ × ♂ (1412.7 × K)	5	74	20	146		
12. (K × 1412.7) ♀ × M ♂	2	0		0		
13. M ♀ × ♂ (K × 1412.7)	3	19	6	103		
14. L ♀ × C ♂	30	10		0	L —	
15. C ♀ × L ♂	24	10		0	C + L —	
15. C ♀ × L ♂	24	10		0	C + L —	
16. M ♀ × C ♂	24	1		8		M — C —
17. C ♀ × M ♂	51	2		2		M —

K = *repleta*, Kilgore; other *repleta* strains by number
L = *limensis*; M = *melanopalpa*, Mexico; C = *canapalpa*
+ = cross fertile; — = cross sterile

When *limensis* females were crossed to *canapalpa* males many of the F_1 larvae died. The wing pads and eye pigment of the 52 viable pupae were visible through the pupa cases, but the adults failed to emerge with the exception of a single female. In one vial of the reciprocal cross, which went exceptionally well, over 134 pupae were counted, most of which failed to extend their horns but had visible wing pads and eye pigment. From the 24 mass matings ten F_2 females were obtained, three of which showed a notched wing effect. Two F_2 females were obtained in a backcross to *canapalpa* males, and these in turn gave fertile F_3 females and males when backcrossed to *canapalpa*.

F_1 males and females were obtained in the reciprocal crosses between *melanopalpa* and *canapalpa*, although most of them showed notched wings and reduced or missing bristles. None of these flies proved to be fertile.

The crosses between *melanopalpa* and various strains of *repleta* were made in an unsuccessful attempt to locate other strains which would yield intersexes. The only point of interest in these crosses is a case of hybridization between a *repleta* female and a *melanopalpa* male which produced a single female offspring.

CYTOLOGICAL OBSERVATIONS

Male and female F_1 larvae were checked from the cross between *limensis* females and *repleta* Kilgore males. Two rearrangements were found, an inversion in the basal portion of the second chromosome and a small inversion in the mid-region of the third. The approximate outer limits of the inversions on Wharton's 1942 map, which we have reproduced on a small scale (Fig. 1), are 2F2d—2G2f and 3D3c—3D5a. Otherwise the chromosomes were usually paired, except near the centromere regions where an obvious difference in the type or amount of heterochromatin was detectable. One of the homologs showed distinct banding while the other was a mass of diffuse-type heterochromatin.

A check of the salivary gland chromosomes of the female hybrid larvae from reciprocal crosses between *limensis* and *canapalpa* revealed three variations in the gene sequences: the inversions in the second and third chromosomes, which were found in the previous cross, and an inversion in the basal portion of the fifth, the approximate limits of which are 5E2g—5F1c. The bases of all the chromosomes showed heterochromatic differences. This condition is pronounced in the X. The sixth chromosomes did not pair in most preparations, one being associated with a mass of heterochromatin and the other with a peculiar clear structure from the *canapalpa* parent. A check of the *canapalpa* stock revealed the presence of a heterozygous inversion, with approximate limits 2D1b—2F2e.

Female larvae from the cross of *melanopalpa* females to *repleta* Kilgore males showed three inversion differences. In the second chromosome the included inversions reported by Wharton (1942) were present, the limits of which have been determined to be at approximately 2C2f—2D5b and 2C6d—2D4c. However, the *melanopalpa* (M) stock is heterozygous for the outer inversion. As this strain of *melanopalpa* has the same gene sequence in the fifth chromosome as *canapalpa*, and *repleta*, like *limensis*, has the normal order, we find the fifth chromosome heterozygous in these hybrids (Figs. 2 and 3). These figures show heterochromatic differences in the bases of some homologous chromosomes, as well as the peculiar clear body associated with the sixth chromosome of *canapalpa* and *melanopalpa*.

The chromosomes of *melanopalpa/canapalpa* hybrids showed only the included inversion in the second chromosome, and all other chromosomes, except the sixth, showed complete synapsis (Fig. 3).

DISCUSSION

Patterson and Stone (1952) have discussed the repleta group in connection with its remarkable specialization of desert adaption for the genus Drosophila. The melanopalpa subgroup is typical in this respect, occurring in the southwestern and Mexican deserts, except for *limensis* from South America and of course *repleta* itself. Using *repleta* as standard, *limensis* L has only the small inversions in the second and third chromosomes and does not share any inversions with *melanopalpa*, *canapalpa* or *neorepleta*, although these latter share a series of inversion differences from *repleta*.

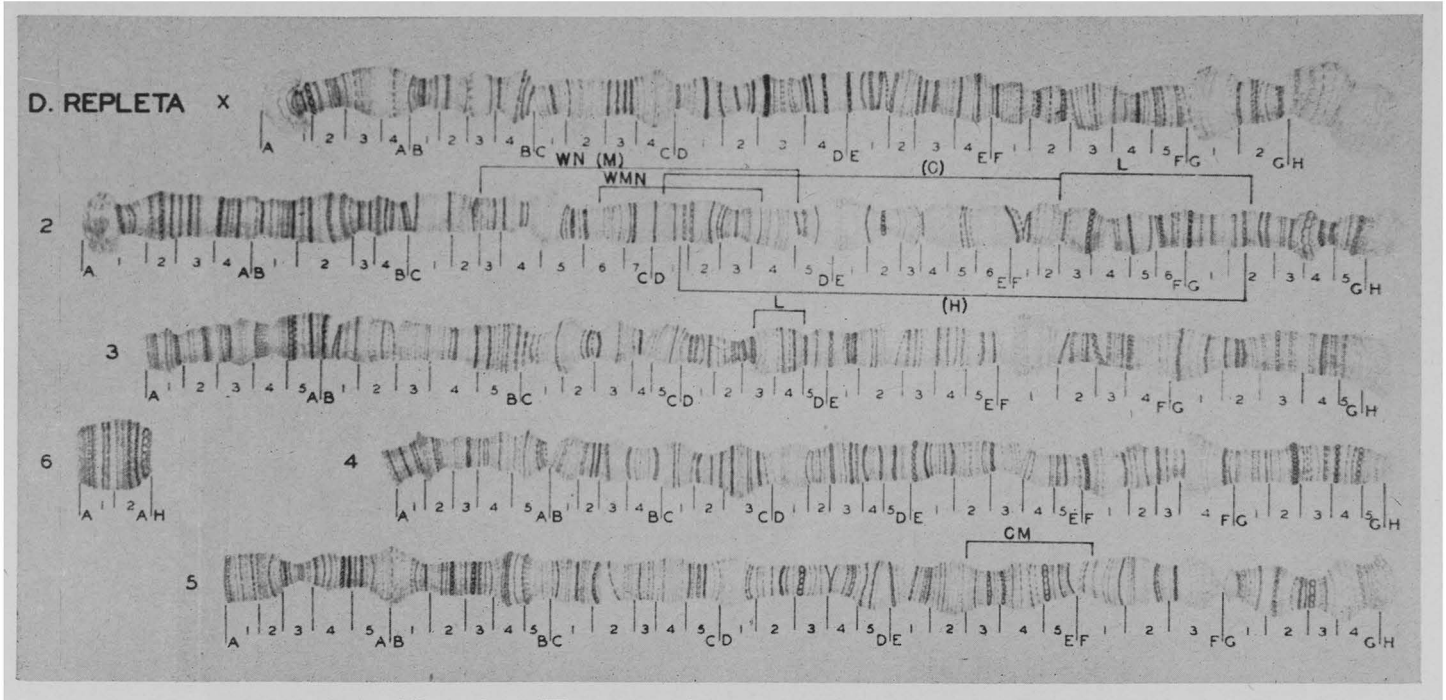


FIG. 1. Chromosome map of *Drosophila repleta* (Wharton 1942). Inversions in the gene sequences of the various species as compared to *D.repleta* are indicated by brackets. The following symbols are used: C, *D.canapalpa;* H, *D.hydei* (Warters); L, *D.limensis;* M, *D.melanopalpa* (Mexico); W, *D.melanopalpa* (Arizona); N, *D.neorepleta.* The letters in parentheses indicate that the inversion is heterozygous in the species.

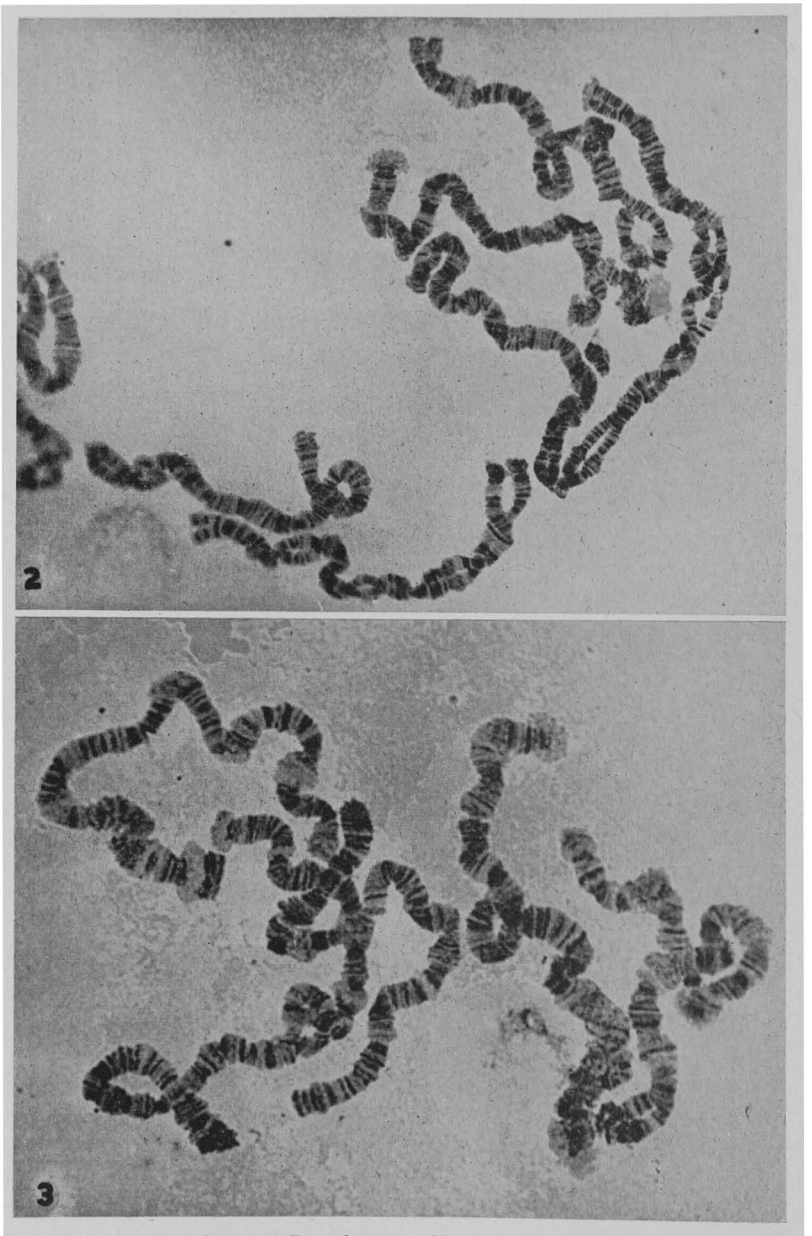


FIG. 2. *D.melanopalpa* ♀ × *D.repleta* ♂. Chromosome 5 (above) has the small basal inversion, and chromosome 2 (right) has the small inversion which is usually included with a larger inversion.

FIG. 3. *D.melanopalpa* ♀ × *D.canapalpa* ♂. Chromosome 2, with the small inversion, is seen in the lower left portion of the photograph.

The genetic tests also show that *limensis* is more closely related to *repleta* or, in other words, that *repleta* is closer to the more primitive form of the group. This situation is similar to that in the virilis group where the widespread species *virilis* is more closely allied genetically and cytologically to the ancestral form. The second chromosome (E element) is variable in these forms. In fact the inversion analyzed in *hydei,* which is present heterozygous in number of strains, involves this element also (Fig. 1). Wharton (1942) found that the two inversions in this element were common to *neorepleta* N and *melanopalpa* W from Arizona. *Drosophila canapalpa* C differs from these forms in that it has the small inversion in the fifth chromosome and the large heterozygous inversion in the second chromosome of which the distal break falls within the limits of the two inversions in *neorepleta.* The Mexican strain of *melanopalpa* M links these forms cytologically for it has the small fifth chromosome inversion characteristic of *canapalpa* and the second chromosome inversions of *neorepleta,* although the larger external inversion is heterozygous only.

Thus the present cytological information would allow us to assume either that the three species came from a common ancestor heterozygous for the three shared inversions which sorted out in the process of species divergence, or conversely, that certain inversions were shared because of introgressive hybridization between species or subspecies giving the present forms. On this basis the *melanopalpa* M from Mexico received its chromosomes from two sources, the ancestors of *canapalpa* and *neorepleta.*

Only a few strains of these forms other than *repleta* have been bred in the laboratory. Wharton (1942) showed that *repleta,* although cytologically homozygous, was genetically heterogeneous. One type of heterogeneity involved sexual isolation factors so that different strains might not cross to each other in one or both reciprocal matings even though both cross to a third strain.

The *repleta* strains used by Wharton and those used in this report are different, but the general results in the crosses to other species are in good agreement. The *repleta* females are almost completely isolated from other species, although one from the Corumba, Brazil, strain gave a single female hybrid to *melanopalpa* M males. In the reciprocal matings to *limensis, canapalpa, neorepleta* and both strains of *melanopala,* both male and female offspring were obtained. Wharton reported that all offspring which she obtained from crosses of *repleta* to *canapalpa, melanopalpa* (Arizona), and *neorepleta* were sterile. However, in these experiments fertile females were produced by the Mexico strain of *melanopalpa Drosophila repleta* males when crossed to *limensis* females produced fertile females and sterile males. A marked effect of genic unbalance is produced in crosses of *repleta* females and *melanopalpa* males. These crosses give an abnormal sex ratio, for approximately twice as many males as females plus female intersexes emerged. Some of the females were fertile in backcrosses to *repleta* but none produced offspring with *melanopalpa.*

All of the intersexes recorded were of the female type in which small claspers were usually seen. The vaginal plates were crossed in some

individuals and the ovaries were undeveloped. In the males which were dissected, the testes were undeveloped. The cross was attempted again using heterozygous *repleta* males, but there was no increase in the number of intersexes produced. This is a parallel case to one described by Wharton who found that *melanopalpa* from Arizona crossed to the Guatemala *repleta* produced male-like, female-like and extremely mixed intersexes, in addition to a few phenotypically normal males and females. In our case the cross produced fewer intersexes and, although abnormalities of the tergites of the progeny were quite common, there were some fertile females. Therefore, there would seem to be less upset of the sex balance than that which occurs when the Arizona strain of *melanopalpa* is crossed to Guatemala *repleta*. The genetic systems have not been analyzed further.

In a similar series of crosses using *neorepleta* and *repleta*, Sturtevant (1946) obtained sterile male progeny, a small number of fertile females and many females which possessed three anal plates instead of two, suggesting intersexuality. By using White, a sex-linked recessive mutant in *repleta*, he was able to demonstrate that a dominant factor responsible for the intersexuality was carried in an autosome of *neorepleta*. There were several interesting reactions involved. The hybrids were backcrossed to the *white* strain repeatedly and the final determinations were made on a minimum number of incompatible factors. The F_1 males from the cross of *neorepleta* females to *white repleta* males are sterile with very narrow testes. With but few exceptions, these males reappear among the phenotypically normal male progeny in the backcrosses to the *white* strain. As the *neorepleta* and *repleta* chromosomes have the same gene sequences except for the inversions in the second chromosome, the factor for narrow testes in the *neorepleta* X is very closely linked to the normal allele of *white* in that chromosome. This is a male cross sterility gene in hybrids, although other genes might also make these hybrid males sterile. It is not the gene which is in balance with the autosomal dominant that causes female intersexuality in these hybrids, for those can be suppressed by another normal gene in the X after the narrow testes gene is crossed out. This separation is difficult as the genes are closely linked.

In the typical backcross from a female having the *neorepleta* X chromosome factor heterozygous with the *repleta* X carrying *white*, and the dominant intersex factor in the *neorepleta* autosome also heterozygous, but with the other chromosomes of *repleta*, all offspring with two *repleta* X chromosomes are intersexes. The heterozygotes that carry the dominant female factor from the *neorepleta* X are again female. Some of these females show signs of intersexuality although part of them are fertile,

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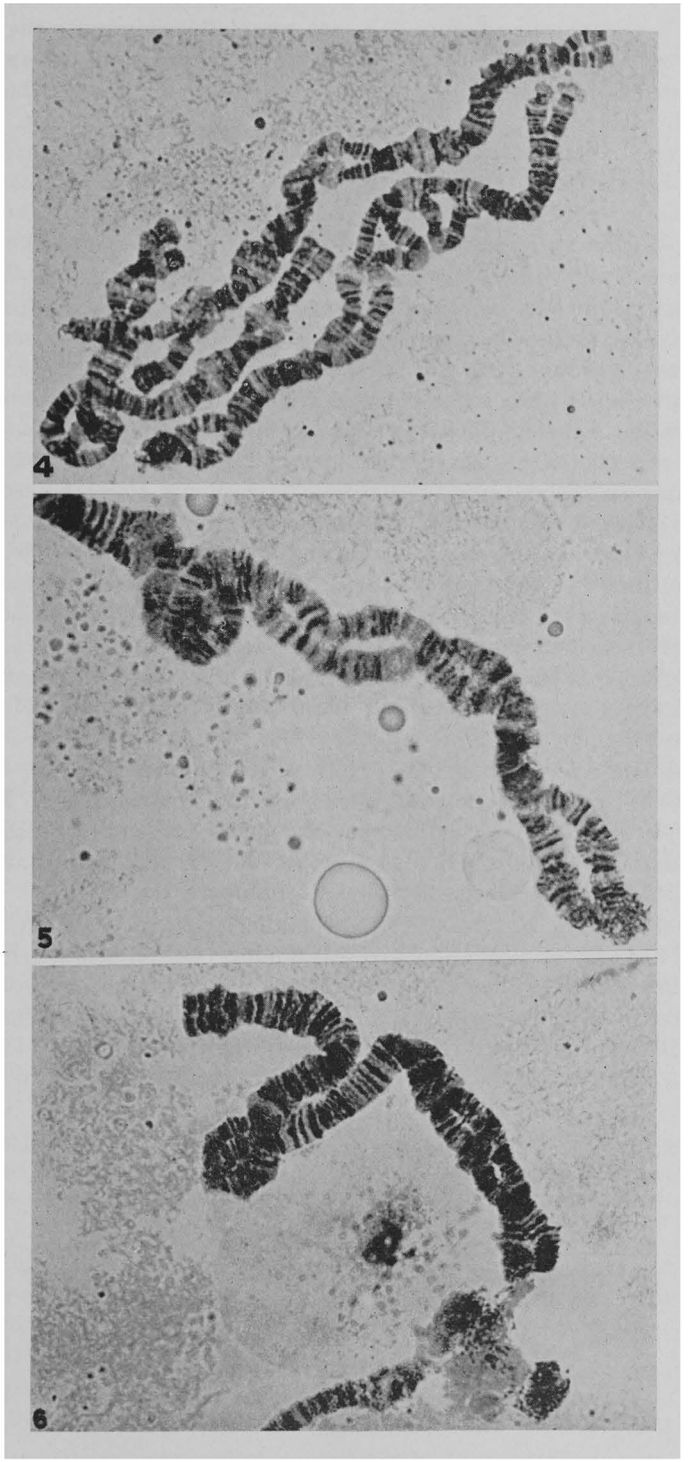
FIG. 4. *D.melanopalpa* ♀ × *D.repleta* ♂. To the right is the included inversion in chromosome 2 and at the lower left, the inversion in chromosome 5.

FIG. 5. *D.melanopalpa* ♀ × *D.repleta* ♂. A portion of chromosome 2 showing the small inversion and the heterochromatic difference at base.

FIG. 6. *D.melanopalpa* ♀ × *D.repleta* ♂. Chromosome 4 is seen associated with the nucleolus and the unpaired 6 chromosomes.


4
5
6

thus the X chromosome factor does not completely dominate the developmental reaction. As the class with both X chromosomes from *repleta* is intersexual, the dominant autosomal factor does not have to be present, but acts by determining the properties of the egg cytoplasm, not, however, just *neorepleta* egg cytoplasm, as these flies can be primarily *repleta* from repeated backcrosses. Furthermore, males *(white)* with *repleta* chromosomes except for the autosome carrying the dominant *neorepleta* intersex factor produce only normal females when crossed to *repleta*. The half of their daughters which carry the *neorepleta* factor produce only XX intersex offspring and males. Thus we have a system where Sturtevant has isolated three primary factors which are responsible for hybrid unbalance. These *neorepleta* factors are in balance with each other to produce normal sexes with the usual *neorepleta* gene and egg cytoplasm system. In the recombination hybrids unbalance results in abnormal sex development. The case of *melanopalpa* and *repleta* may be related, as some strains of the former and *neorepleta* give fertile male and female hybrids according to Wharton. Also *neorepleta* and *melanopalpa* W showed strong sexual isolation with *canapalpa*, but the few progeny obtained were fully fertile. We were able to obtain only a few hybrids, which were usually abnormal, between *canapalpa* and *melanopalpa* M and these proved to be sterile.

With rare exceptions, crosses between *canapalpa* and *limensis* produce zygotes which are larval and pupal lethals. The pupae often showed wing pads and eye pigment but were unable to emerge. The cross of this last species to *melanopalpa* M gave no offspring.

In these latter cases the genic unbalance is very pronounced, as is sexual isolation, but produces abnormal, sterile or lethal combinations. The cross lethal effect is presumably autosomal and dominant, and, unlike the sex-linked *aldrichi 2* cross-lethal factor in the mulleri subgroup analyzed by Crow (1942), is not open to simple genetic analysis.

The melanopalpa subgroup and the mulleri subgroup which has been analyzed further by Patterson and Alexander (see Article VIII) are excellent examples of extensive species groups where the major evolutionary divergence is genetic rather than chromosomal, and in several cases depends on the interactions of a few dominant factors.

REFERENCES

Crow, J. F. 1942. Cross fertility and isolating mechanisms in the mulleri group. Univ. Tex. Publ., 4228:53–67.

Patterson, J. T., and W. S. Stone. 1952. Evolution in the genus Drosophila. Macmillan Co., New York (in press).

Sturtevant, A. H. 1946. Intersexes dependent on a maternal effect in hybrids between *Drosophila repleta* and *D.neorepleta*. Proc. Nat. Acad. Sci., 22:448–450.

Warters, Mary. 1944. Chromosomal aberrations in wild populations of Drosophila. Univ. Tex. Publ., 4445:129–174.

Wharton, L. T. 1942. Analysis of the repleta group of Drosophila. Univ. Tex. Publ., 4228:23–42.

Wharton, L. T. 1944. Interspecific hybridization in the repleta group. Univ. Tex. Publ., 4445:175–193.

VIII. *DROSOPHILA WHEELERI*, A NEW MEMBER OF THE MULLERI SUBGROUP

J. T. PATTERSON AND MARY L. ALEXANDER

INTRODUCTION

On a collecting trip into Sonora, Mexico, in 1941, G. B. Mainland and R. P. Wagner collected fifty-five specimens of a Drosophila species in a cactus patch located three miles west of Hermosillo (August 24), and on the same day four additional specimens were taken in a similar patch about three miles north of the same city. These flies were listed in their field notes as "aldrichi-like." The specimens proved difficult to maintain in stock in the laboratory, and the culture was soon lost. Although this form was recognized as differing from *Drosophila aldrichi* of Patterson and Crow 1940, nevertheless, its distribution and collection records were later listed under that name (Patterson and Mainland 1944; Map 12, p. 76, and Table 8, p. 94).

This form was not seen again by us until the spring of 1950, when Dr. M. R. Wheeler sent a stock to this laboratory from Pasadena, California. The stock originated from a pair of flies taken late in October, 1949, in traps by Professor W. P. Spencer at the Arboretum, Arcadia, California. It was soon found by one of us (Alexander) that the stock could be maintained if an extract from the fruit of the common cactus was added to the food medium. It was thus possible to study and compare the morphology of this form with those of *aldrichi* and certain other members of the mulleri subgroup, to which it clearly belonged. As a result of such studies, together with genetic tests, we have reached the conclusion that it represents a new species, to which we have given the name *Drosophila wheeleri* in honor of Dr. Marshall R. Wheeler.

In lieu of a full description of the new species, we present below two series of characters, the first of which includes those that are common to both *aldrichi* and *wheeleri*, and the second in which the characters differ in the two species. The characters for *aldrichi* are based on the description of this species by Patterson and Wheeler (1942).

Characters different in the two species

Arista with 7 branches, 3 above and 2 below in addition to terminal fork
Carina broad and sulcate
Cheeks yellowish-gray
Acrostichal hairs in 8 rows, no prescutellars
Anterior scutellars convergent
Abdomen pale yellow, each segment with an interrupted brown band
Crossveins not noticeably clouded
Spermatheca spherical, not sclerotized
Ventral receptacle poorly formed and without distinct coiling

Chromosomes, 5 pairs of rods and one pair of dots; Y shorter than X
Length of body in live specimen, 2.5 mm.

Characters different in the two species

D. aldrichi	*D. wheeleri*
Cheeks ¼ greater diameter of eyes	⅓ greatest diameter
Eyes vermilion-like	dark red
Mesonotum grayish-brown	light tannish-brown
Wings clear, veins brown	with faint grayish overcast
Heavy bristles on basal ¼ of 3rd costal section	on basal ⅓
Dark lateral area at ventral edge of tergite connected with central band in posterior segments	not connected with central band
Testes deep orange	pale yellow, darker in old flies
Puparium orange-tan, anterior spiracle with 9 branches, horn-index 2.3	pale yellow, 9 branches, horn-index 2.9
Distribution range, Texas and eastern Mexico	California and Sonora

Types.—Holotype male and nine paratypes (No. 1980.1), descendants of the original pair from Arcadia, California, placed in the collection of The University of Texas.

These comparisons make it possible to separate the two species, especially in live specimens. The simplest character difference to use is seen in the abdominal color pattern. This difference is clearly brought out in Figure 1.

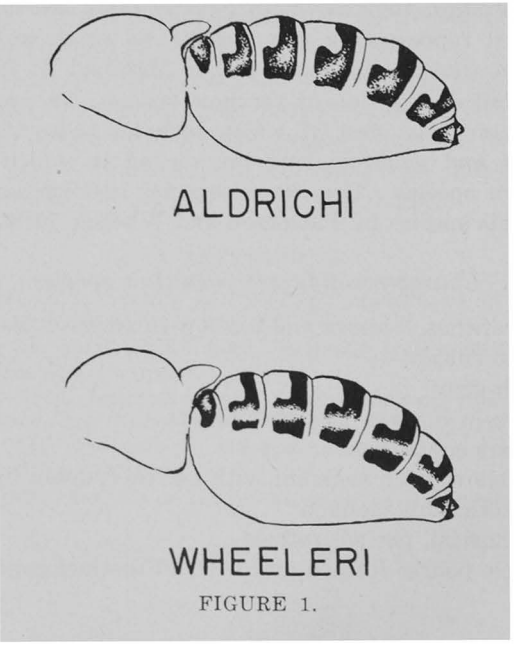


FIGURE 1.

CROSSES BETWEEN *WHEELERI* AND FOUR OTHER MEMBERS OF THE SUBGROUP

In the first series of tests, reciprocal crosses in mass matings between *wheeleri* and four other members of the subgroup were carried out. The four species used were *mulleri* (Mi), *mojavensis* (Mo), *arizonensis* (Az) and *buzzatii* (B). In the first reciprocal cross with *mulleri* used as the male parent, 200 tested pairs gave 28 females and no male hybrids, and with *wheeleri* as the male parent, the same number of tested pairs yielded 54 females and 71 males (Table 1, 1 and 2). In the second pair of crosses, with *mojavensis* used as the male parent, 250 tested pairs gave 42 female and no male hybrids, and in the reciprocal cross, 150 tested pairs gave but three hybrids, all males (3 and 4). In the third pair of matings, with *arizonensis* as the male parent, 150 tested pairs produced nine weak female hybrids and no males, while the reciprocal cross was incompatible (5 and 6). Finally, in the fourth pair of matings, in the W♀ × B♂ cross, 300 tested pairs yielded 46 pupae from which only six bobbed-like F_1 females emerged, five of these died within a day, leaving a single specimen to be tested for fertility. In the reciprocal cross, B♀ × W♂, no offspring was produced (7 and 8).

In the fourth and fifth columns of Table 1 are listed the results obtained in the tests for the fertility of the F_1 hybrids. The 57 tested male hybrids

TABLE 1

Hybridization between *Drosophila wheeleri* and four other members of the mulleri subgroup*

	Crosses ♀ ♂	Number tested in masses	F_1 offspring produced ♀	♂	Number of F_1's tested ♀	♂	Number of F_1's fertile ♀	♂
1.	W × Mi	200	28	0	18	....	0	....
2.	Mi × W	200	54	71	35	54	2	0
3.	W × Mo	250	42	0	24	....	0	....
4.	Mo × W	150	0	3	....	3	....	0
5.	W × Az	150	9	0	2	....	0	....
6.	Az × W	150	0	0	....	....	....	....
7.	W × B	300	6	0	1	....	0	....
8.	B × W	300	0	0	....	....	....	....

*Symbols used: A, *aldrichi;* Az, *arizonensis;* B, *buzzatii;* Mo, *mojavensis;* Mi, *mulleri;* W, *wheeleri.*

all proved to be completely sterile. Of the 80 tested female hybrids only two showed any fertility. The detailed results from the backcross tests of the hybrid females are presented in Table 2. There was considerable mortality among the hybrids, thus reducing the number available for such tests. Moreover, they continued to die after the beginning of the tests (c.f., column two with columns three and four). The data on the numbers of females tested, with mature eggs, inseminated, producing pupae and adults are given in columns two to six, respectively.

For the first pair of crosses, only 18 of the 28 WMi females were available for testing, and of this number, ten were mated to *mulleri* males

and eight to *wheeleri* males. Both matings were incompatible (1, 2). In the second pair of crosses, the 19 MiW females mated to *mulleri* males gave three pupae, from two of which sterile females emerged, but the 16 crossed to *wheeleri* males failed to produce offspring (3, 4). In the third pair of matings, the 13 WMo females crossed to *mojavensis* males gave two pupae, while the cross of 11 to *wheeleri* males was incompatible (5, 6). Of the two WAz females, one was mated to *arizonensis* males and the other to *wheeleri* males, but both crosses were incompatible (7, 8). The cross between the single WB female and *buzzatii* males was incompatible (9).

TABLE 2

First backcross of hybrid females

Crosses ♀ ♂	Number tested	Number with mature eggs	Number inseminated	Number producing pupae	Number producing adults
1. WMi × Mi	10	3 in 6	0	0	0
2. WMi × W	8	2 in 3	3 in 3	0	0
3. MiW × Mi	19	11 in 15	4 in 15	3	2
4. MiW × W	16	9 in 10	2 in 10	0	0
5. WMo × Mo	13	4 in 11	11 in 11	2	0
6. WMo × W	11	2 in 7	4 in 7	0	0
7. WAz × Az	1	1 in 1	1 in 1	0	0
8. WAz × W	1	1 in 1	1 in 1	0	0
9. WB × B	1	0 in 1	0 in 1	0	0
Totals	80	33 55	26 49	5	2

The total number of females tested was 80, of which 33 of the 55 examined had mature eggs in their ovaries (60%), with the remaining 22 possessing underdeveloped gonads. Twenty-six of the 49 dissected females had been inseminated (53%). Of the 80 hybrid females only five produced pupae and from two of these sterile females emerged. While these numbers seem small, nevertheless, they show that at least three isolating mechanisms are present. In the first place, sexual isolation is partly responsible for the failure to yield a larger number of offspring. This is indicated by the fact that nearly one-half of the females had not been inseminated. In the second place, while 26 of the females had been fertilized, yet only two produced adult flies, indicating that the effects of the insemination reaction had prevented the production of a larger number. It has been demonstrated that this reaction is a very potent barrier to crossfertility among members of the mulleri subgroup (Patterson, 1947). Finally, hybrid sterility represents a third type of isolating mechanism among these hybrids. Not only were all of the male hybrids completely sterile, but a large number of the female hybrids were also sterile and frequently showed underdeveloped gonads. The possibility for gene exchange to occur in nature between *wheeleri* and the other four tested forms would be extremely remote, even though they should occupy the same area.

CROSSES BETWEEN *WHEELERI* AND *ALDRICHI*

In the preceding section the results from the crosses between *wheeleri* and four other members of the subgroup were presented. In the present section the data on the crosses between *wheeleri* and *aldrichi* will be given and analyzed. In this series pair matings were used in all crosses with the exception of the four backcross tests of the F_1 hybrid males, where mass matings were employed (Table 3, 9–12). In the control tests the A × A cross gave a lower fertility percentage but a higher average hatch per vial than the W × W cross (Table 3, 1, 2). In the P_1 crosses the percents fertile, as well as the average hatch per vial, was higher when *aldrichi* entered the cross as the male parent (Table 3, 3, 4). In the

TABLE 3

Crosses between *D. wheeleri* and *D. aldrichi*

Crosses ♀ ♂	Pairs tested	Pair fertile	%	Average hatch per vial	Sex-Ratio ♀	♀
Controls						
1. A × A	130	89	68.4	50.1	253	248
2. W × W	105	81	77.1	43.0	217	213
P_1 crosses						
3. A × W	84	55	65.5	34.6	842	817
4. W × A	86	73	84.9	42.7	1167	1181
F_1 backcrosses						
5. AW × A	95	63	66.3	63.4	1301	1425
6. AW × W	96	68	70.8	46.5	897	824
7. WA × A	81	35	42.2	44.8	674	669
8. WA × W	98	80	81.6	61.1	1387	1363
9. A × AW	Mass	0				
10. W × AW	Mass	0				
11. A × WA	Mass	0				
12. W × WA	Mass	0				
F_2 inbred						
13. (AW)A × (AW)A	99	6	6.06	9.0	22	14
14. (AW)W × (AW)W	101	3	2.97	43.3	68	62
15. (WA)A × (WA)A	102	5	4.90	29.0	72	73
16. (WA)W × (WA)W	108	10	9.26	50.5	103	99
F_2 backcrosses						
17. (AW)A × A	97	58	59.8	27.68	279	330
18. (AW)A × W	93	60	64.5	18.1	422	340
19. A × (AW)A	103	5	4.9	23.3	52	41
20. W × (AW)A	96	2	2.08	2.5	3	2
21. (AW)W × A	94	66	70.2	57.6	1070	1006
22. (AW)W × W	85	51	60.0	37.6	521	494
23. A × (AW)W	99	0	< 1.0			
24. W × (AW)W	86	3	3.49	2.0	1	3
25. (WA)A × A	93	70	75.3	44.3	1059	935
26. (WA)A × W	85	49	57.6	25.2	373	384
27. A × (WA)A	97	6	6.19	38.5	133	98
28. W × (WA)A	93	8	8.6	27.6	64	74
29. (WA)W × A	88	67	76.1	49.7	972	917
30. (WA)W × W	85	53	62.4	42.8	721	691
31. A × (WA)W	94	0	< 1.0			
32. W × (WA)W	96	3	3.1	24.5	27	22

backcross tests of the F_1 hybrids, both types of females were fertile to the two types of males (5–8), but the F_1 males proved to be completely sterile. Both the AW and WA females gave higher percentages of fertility in backcrosses to *wheeleri* males than when mated to *aldrichi* males.

In the inbred tests of the F_2's the percents fertile were low, ranging from 2.97 to 9.26, although three out of four of the crosses showed a relatively high average hatch per vial (13–16).

The results from the F_2 backcross tests are listed in the lower half of Table 3, crosses 17–32. Analysis shows that in the eight matings of the F_2 females to *aldrichi* and *wheeleri* males the percents fertile and the average hatch per vial for all eight crosses (except for the percent in cross 15) were higher when *aldrichi* was used as the male parent. These averages were 70.3% versus 61.1% and 44.8 versus 31.2 average per hatch for *aldrichi* and *wheeleri*, respectively.

In the backcrosses of the F_2 males the percents fertile were very low, and in two of the crosses (23 and 31) the percent was less than one. In contrast to this, the average hatch per vial is relatively high; e.g., in crosses 19, 27, 28 and 32. One point of interest is the fact that certain of the male hybrids had degenerate testes. In both P_1 crosses some of the male hybrids had such testes. In the F_1 backcrosses, in which *wheeleri* represented the male parent, some F_2 males also had degenerate gonads.

DISCUSSION AND CONCLUSIONS

In order to bring out the possibilities of gene exchange and relationships between these six members of the mulleri subgroup, we have constructed Table 4. The greater part of these data is from a previous publication

TABLE 4

P_1 crosses between six members of the mulleri subgroup

♀ ♂	*aldrichi*	*arizonensis*	*buzzatii*	*mojavensis*	*mulleri*	*wheeleri*
aldrichi	.....	st. ♀ ♀	none	st. ♀ ♀	none	ft. ♀ ♀, st. ♂ ♂
arizonensis	none	.....	larvae	ft. ♀ ♀, st. ♂ ♂	none	none
buzzatii	none	none	.....	none	none	larvae
mojavensis	none	ft. ♀ ♀ & ♂ ♂	none	.....	none	st. ♂ ♂
mulleri	st. ♀ ♀ & ♂ ♂	st. ♂ ♂	ab. flies	ft. ♀ ♀, st. ♂ ♂	.....	ft. ♀ ♀, st. ♂ ♂
wheeleri	ft. ♀ ♀, st. ♂ ♂	st. ♀ ♀	ab. ♀	st. ♀ ♀	st. ♀ ♀	.....

(Patterson, 1947), the rest is from the present article. A fuller account covering these two points for the first five species listed will be found in the 1947 article, pp. 35–36. In the present article we are mainly concerned with the relationship of *wheeleri* to the other five species. The data show that *aldrichi* could exchange genes with *wheeleri* through the female line, since both reciprocal crosses produce at least some fertile female hybrids. Obviously, *arizonensis* and *mojavensis* could exchange genes. As for *buzzatii*, no exchanges are possible, either because of the incompatibility of the crosses, or because of the abnormality and sterility of the hybrid zygotes.

Furthermore, this species is geographically isolated from all of the other species. Gene exchanges are possible between *mojavensis* and *mulleri*, and between *mulleri* and *wheeleri*, all through the female lines. Finally, *wheeleri* could exchange genes with *aldrichi*, and, of course, with *mulleri*.

These suggested possibilities are based on the results from genetic tests carried out in the laboratory, but for such exchanges to occur in nature, any two species producing fertile hybrids would have to occupy overlapping distribution ranges. On the basis of the known distribution records, *aldrichi* and *wheeleri* are isolated geographically, as are *mulleri* and *wheeleri*. As for *mulleri* and *mojavensis*, they too have geographic isolation. This leaves *arizonensis* and *mojavensis* to be considered. It is possible that the ranges of these two species overlap at some points in western United States, but thus far we have no records indicating that they do. There are a few cases of overlapping distribution ranges of two or more species which produce sterile or abnormal offspring. For example, *mulleri* and *aldrichi* are sympatric over a wide area in Texas and northern Mexico, and *arizonensis* and *wheeleri* have both been collected in the vicinity of Hermosillo in the state of Sonora, Mexico. A few specimens of *arizonensis* have been collected in the state of Tamaulipas, Mexico, in close association with both *aldrichi* and *mulleri*. These different species are able to live together without the loss of identity, due to the development of complete reproductive isolation.

The possibility of some type of maternal influence is seen in the backcrosses of the F_1 females to *wheeleri* and *aldrichi* males. In crosses 5 and 8, where the mating involves males of the same type as the original P_1 female, the averages for the hatch per vial are 63.4 and 61.1, respectively. When the males were not of the same species as the original female, lower averages of 46.5 and 44.8 were obtained (crosses 6 and 7). Some of the female offspring (F_2) of the latter two crosses were found to have degenerate or no ovary differentiation. Such individuals were detected only among the offspring from the paired matings and were not found among those from mass matings. The detection of any effect in the F_2 backcross is not probable since all individuals used were from F_1 mass matings. The separation of this type of influence from that of homozygosity for genes of one species in the F_2 individuals would be difficult. It is also possible that some pairs produced females with degenerate gonads, whereas others did not. Therefore, there is clearly a reduction in the average hatch per vial when a male foreign to the original female is crossed to the F_1 hybrid females, but the exact nature of the production of immature and undifferentiated ovaries can not be determined without additional tests.

Isolation between members of the subgroup was rather complete when the crosses involved *mulleri* males and only one, *wheeleri* females $\times$ *mulleri* males, produced offspring, although such males did inseminate females of *aldrichi*. In this respect *wheeleri* differs from all the other members. The genetic results clearly indicate that *wheeleri* is more closely related to *aldrichi* than to any other species, with *mulleri* the next nearest relative.

REFERENCES

Patterson, J. T., and J. F. Crow. 1940. Hybridization in the mulleri group of Drosophila. Univ. Tex. Publ., 4445:251–256.

Patterson, J. T., and G. B. Mainland. 1944. The Drosophilidae of Mexico. Univ. Tex. Publ., 4445:9–101.

Patterson, J. T., and M. R. Wheeler. 1942. Description of new species of the subgenera Hirtodrosophila and Drosophila. Univ. Tex. Publ., 4213:67–109.

Patterson, J. T. 1947. The Insemination Reaction and its Bearing on the Problem of Speciation in the Mulleri Subgroup. Univ. Tex. Publ., 4720: 41–77.

IX. CHROMOSOME VARIATION IN DROSOPHILA MELANICA

CALVIN L. WARD

INTRODUCTION

A new method of approach to the problem of speciation was opened with the rediscovery of the salivary gland chromosomes of Drosophila by Painter (1933). Previously it had been possible to analyze inversions only by long genetic experiments such as those reported by Sturtevant in 1931, but with Painter's work it became possible to detect and analyze rearrangements in the gene sequence by simple cytological methods, and to determine both their size and position.

Since that time extensive investigations have been carried out on various species of Drosophila in attempting to determine the presence, distribution, and significance of chromosomal aberrations in wild populations. In some few cases it has been possible to work out the phylogeny of the gene sequences, and attempts have been made to explain the retention and fixation of these rearrangements in wild populations. That chromosomal aberrations are widespread in nature was shown by Dubinin, Sokolov, and Tiniakov (1936, 1937). *Drosophila pseudoobscura* has been investigated extensively by Dobzhansky and his collaborators, and the data on chromosomal rearrangements in this and the closely related species *D. persimilis* were summarized by Dobzhansky and Epling (1944). Other investigations of inter- and intraspecific chromosome variation in wild populations include the work on *D. ananassae* by Kaufmann (1936), Kikkawa (1938), and Dobzhansky and Dreyfus (1943); on *D. algonquin* by Miller (1939); on *D. azteca* by Dobzhansky and Sokolov (1939) and Dobzhansky (1941a); on *D. athabasca* by Novitiski (1946); and on *D. robusta* by Carson and Stalker (1947). The investigation of the cytology of the virilis group has been carried out by Hughes (1939), Patterson, Stone and Griffen (1940, 1942) and more extensive work on the chromosomal variation, by Warters (1944) and Hsu (this publication). In addition to these, other investigations have been published in which it has been found that the amount of variation in gene sequences varies greatly. In some species, such as *D. virilis*, geographically remote strains showed no differences in gene sequence (Warters, 1944), and *D. repleta* was found to have a very stable gene sequence (Wharton, 1942). The other extreme has been found in *Drosophila willistoni* where forty different inversions have been recorded (Da Cunha, Burla, and Dobzhansky, 1950).

On the basis of a report by Griffen (1942), it seemed probable that *D. melanica* would yield interesting chromosomal variability and therefore a cytological investigation has been undertaken on this species.

MATERIALS AND METHODS

The materials used in this work consisted of sixty-two strains of *D. melanica melanica* Sturtevant which are listed according to strain number

TABLE 1

Chromosome Variability in *Drosophila melanica*

Strain	Locality	XR	XL	2	4
36.3c	Bastrop, Tex.	+	+	+	+
74.4	Georgetown, Tex.	+	a	+	+
1500.8d	Austin, Tex.	ab	+	+	+
1502.5	Austin, Tex.	ab	+	+ degh	+
1504.6a	Austin, Tex.	ab	+	+	b
1589.5	Morrilton, Ark.	ab	+	+ deghi	+
1713.4	Apache Refuge, N. M.	ab	+	+ degh	+
1714.4e	San Antonio, N. M.	ab	+	+ degh	+
1720.3	Cliff, N. M.	ab	+	+ degh	+
1728	Las Delicias, Chihuahua, Mex.	ab	+	degh	+
1750.3	Medicine Park, Okla.	ab	+	adeghj	+ a
1751.4	Wichita, Kans.	ab	+	b bdeghj kndeghj	+ b
1760.2	Poplar, Mont.	ab	+	degh	+
1761.2	Chinook, Mont.	ab	+	degh bdegh	+ a
1875.6	Demopolis, Ala.	ab	+	+ l bdefgh	+
1878.2	Albemarle, N. C.	ab	+	bdegh cdghop	+
1880.10	Richmond, Va.	ab	+	bdegh cdgho	+ b
1906.8	Carlinville, Ill.	ab	+	+ bdefgh	+
1910.7	Dexter, Mo.	ab	+	+ bdefgh cdgho	+
1952.6	Antlers, Colo.	ab	+	+ bdegh	+
1954.5a	Whitewater, Colo.	ab	+	+ bdegh	+
1954.5b	Whitewater, Colo.	ab	+	+ bdegh	+
1954.5c	Whitewater, Colo.	ab	+	degh bdegh	+
1954.5d	Whitewater, Colo.	ab	+	+ degh	+
1954.5e	Whitewater, Colo.	ab	+	+	+
1954.5f	Whitewater, Colo.	ab	+	+ bdegh	+
1954.5g	Whitewater, Colo.	ab	+	+ bdegh	+
1954.5h	Whitewater, Colo.	ab	+	bdegh	+
1958.3a	Pojuaque, N. M.	ab	+	+ degh	+
1958.3b	Pojuaque, N. M.	ab	+	+ degh	+
1958.3c	Pojuaque, N. M.	ab	+	degh bdegh	+
1958.3d	Pojuaque, N. M.	ab	+	+ degh	+
1958.3e	Pojuaque, N. M.	ab	+	+	+
1970.5	Carbondale, Ill.	ab	+	bdeghi cdgho	+
1973.5	Texarkana, Tex.	ab	+	+ bdegh	+
1978.1	Cedar Park, Tex.	+	+	+	+

TABLE 1—Continued

Chromosome Variability in *Drosophila melanica*

Strain	Locality	XR	XL	2	4
1978.2	Walnut Creek, Ariz.	ab	+	+	+
2005.4	DeSoto Forest, Miss.	bc	+	cdgho acdgho	+
2007.7h	Tallahassee, Fla.	b ab	+	+	+
2008.9	Myakka Head, Fla.	b bc	+	+ acdgho	b
2011.3	Sanford, Fla.	bc	+	+ acd acdgho	b
2016.5	Acworth, Ga.	ab	+	degh bdefgh kbdefgh	+
2020.7b	Tombigbee Park, Miss.	ab	+	degh kndegh cdgho amdh	+ a
2067.13	Chadron, Nebr.	ab	+	kdegh kdefgh bdefgh	+ b
2068.8	Oakdale, Nebr.	ab	+	+ degh kdegh	+
2068.8a	Oakdale, Nebr.	ab	+	+ bdefgh	+ b
2068.8c	Oakdale, Nebr.	ab	+	+ degh deghi kdegh kdeghi	+ b
2069.14	Hastings, Nebr.	ab	+	kndegh bdegh bdeghi bdefgh	+ b
2070.8	Haigler, Nebr.	ab	+	+ degh kdegh bdefgh	+ b
2070.8a	Haigler, Nebr.	b ab	+	degh bdefgh	+ b
2070.8b	Haigler, Nebr.	ab	+	kdegh kndegh	b
2070.8c	Haigler, Nebr.	ab	+	kdegh	+
2070.8e	Haigler, Nebr.	ab	+	kdegh	+ a b
2073.3	Albuquerque, N. M.	ab	+	degh bdegh	+
2073.3a	Albuquerque, N. M.	ab	+	degh	+
2073.3c	Albuquerque, N. M.	ab	+	+ degh bdegh	+
2073.3d	Albuquerque, N. M.	ab	+	degh bdegh	+
2073.3e	Albuquerque, N. M.	ab	+	degh bdegh	+
2075.2	Cliff, N. M.	ab	+	+ degh adh	+
2075.2a	Cliff, N. M.	ab	+	degh adh	+
2075.2c	Cliff, N. M.	ab	+	bdegh	+
2076.7a	Silver City, N. M.	ab	+	+ degh bdegh	+

and locality in Table 1. The homozygous stock from Bastrop Park, Texas was chosen as the standard strain, and all crosses were made, using the Bastrop females and the males of the strain to be tested. Small mass matings of ten pairs of flies were made and transferred weekly to insure numerous, well fed larvae. Ten female larvae from each cross were dissected and their chromosomes examined.

Slides were prepared using the following technique: the salivary glands were dissected from the larvae and immediately transferred to N HCl for several minutes. They were then removed to 50% solution of aceto-orcein for several minutes, and finally squashed in 50% acetic acid. All preparations were temporary mounts made by ringing the coverslip with a mixture of paraffin and vaseline. Slides prepared in this manner will last for several weeks, making it possible to recheck when necessary.

The chromosome map for *D. melanica* is a composite of numerous drawings made with the aid of a camera lucida (Fig. 1).

RESULTS

Ten elements may be seen in the brain smear; two pairs of V-shaped chromosomes, two pairs of rod-shaped chromosomes, and one pair of dots. The smaller pair of V's is autosomal and resulted from a pericentric inversion in a rod-shaped chromosome. The large V is the X chromosome, and the Y is J-shaped. In the salivary gland preparations there are present four long arms two medium length arms and one very short arm. Two of the long arms are haploid in the male and the fused pair represents the X chromosome. The two remaining long arms correspond to the two pairs of rods seen in methaphase, and the two medium length arms to the pair of small V-shaped chromosomes.

The chromosome map of *D. melanica* (Fig. 1) was drawn, using the strain 36.3c from Bastrop, Texas. The system for designating the chromosome bands on this map is that first used by Griffen (Patterson, Stone and Griffen, 1940) in which each chromosome is divided into eight sections using the letters A through H from the free end to the centromere, with section H including only the heterochromatin. Each large section is then divided into subdivisions, ranging from one to nine, which are designated by arabic numerals. The bands within each of these smaller divisions are designated by small letters, starting at the left end of the section and reading alphabetically to the right. Doublets are given a single designation.

The right arm of the X chromosome is characterized by a large puff at the free end. This arm has five conspicuous weak points along its length, two of which are located between the two basal puffs of the chromosome. These puffs probably represent regions easily disrupted by the acid treatment. A large mass of darkly staining heterochromatin is seen at the base of this strand near the point at which it is fused with the left arm.

The left arm of the sex chromosome exhibits a weak point very near to the tip of the chromosome, and it is usually impossible to distinguish the

banding in the short terminal region. This arm is also characterized by five weak points and conspicuous bulbs or puffs in the regions of D3, E2, and F1. The darkly staining doublet in E1 is an excellent landmark.

Chromosome 2, the longest element in *melancia,* has an easily recognized tip marked by two darkly staining bands which are followed by three light and then five heavily stained bands. The double puffs in region C are easily recognized. In region D3 there are three bands which, while not darkly staining, are always quite easily located. The short section, F1 and 2, is easily identified since it is bounded by weak points. There is a single fuzzy band of heterochromatin at the centromere end of this chromosome.

Chromosome 3 may be recognized by the slight puff near the free end and the two darkly staining bands on the tip. Points C, F, and G locate weak spots, that at C being particularly distinguishing because it is preceded and followed by enlargements.

The centromere of chromosome 4 is located at BC which is also a weak point, making a total of nine such locations in this chromosome. The right arm of this element is characterized by a slight flare followed by four dark bands and a large puff.

A total of twenty-two inversions was found in the chromosomes of *D. melanica,* three in the right arm of the X chromosome, one in the left arm, sixteen in 2 (longest element, presumably E), none in 3 and two in the right arm of chromosome 4. The inversions are designated by letters following the name of the chromosome in the sequence in which they were found, such as 2*a*, 2*b*, etc. The inversions are diagrammed in Figure 2, and in the following descriptions the location of the break points are given. In some instances these locations are exact, but the majority must be considered as approximate and may eventually be moved by several bands when determined more exactly.

Inversion XR *a* is a large rearrangement involving more than a third of the length of this arm. Its approximate limits are XRB4b—XRF1b. Inversion *b* (XRB4f—XRD2b) may occur separately or within inversion *a*, and a small inversion *c* (limits undetermined) was found in region F of some *b* strains but not in *a b* strains. Only one inversion has been found in the left arm of the X chromosome and its limits are XLE1b—XLF3e.

Inversion 2 *a* is located in the terminal region of the chromosome, the limits are at 2A1b—2A5a. When this inversion is heterozygous with the standard gene sequence, it forms a loop in which the terminal two bands of the chromosome do not pair (Fig. 12). Inversion 2 *b* (2A4e—2B3c) is slightly shorter than *a* and more proximal in position.

Inversion 2 *c* is a rather large rearrangement extending from 2B2b to 2C5g that always includes the small inversion *d*. Inversion 2 *d* is small and includes one of the very characteristic regions of chromosome 2 which shows two large puffs. The outer limits of this inversion have been located at 2B6g and 2C4c. Inversion 2 *e* is about the same length as *d*, and

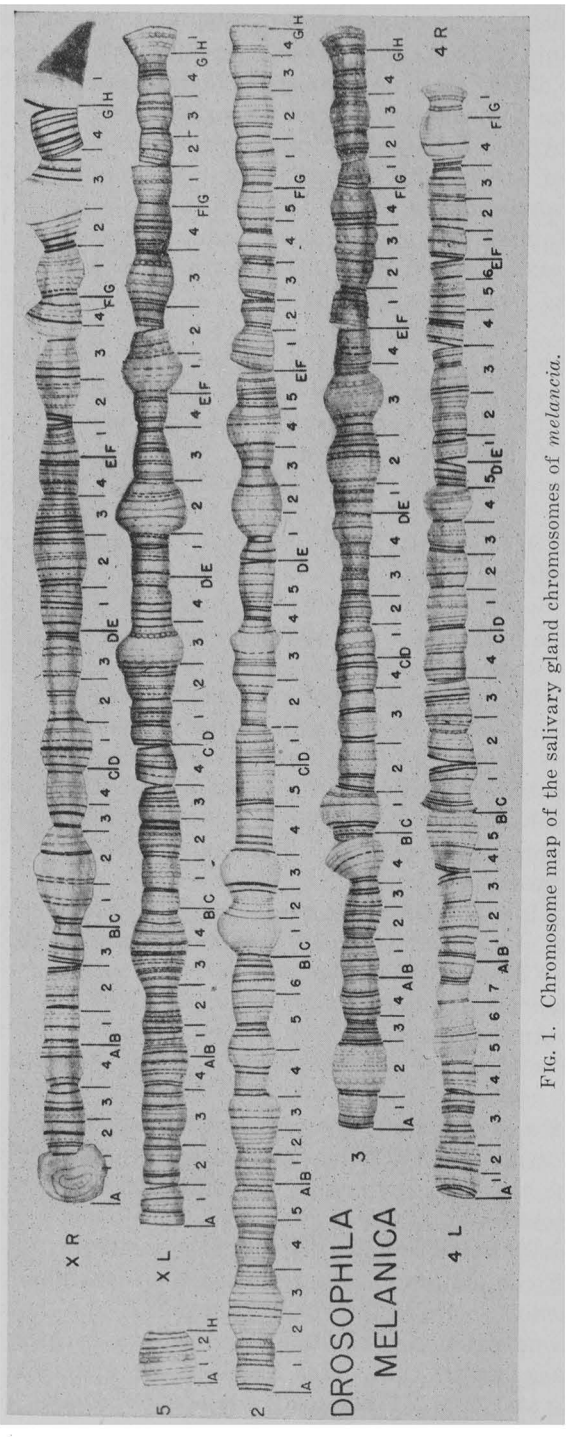


FIG. 1. Chromosome map of the salivary gland chromosomes of *melancia*.

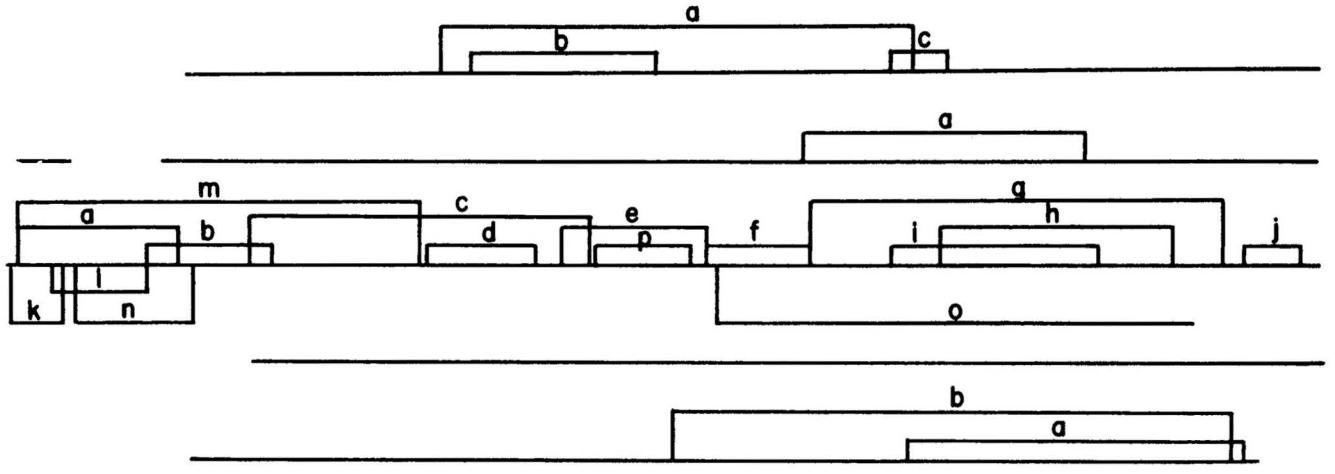


FIG. 2. Diagram of inversions in *melancia*, with chromosomes arranged in the same order as in Figure 1.

its limits are at 2C4f and 2D3b. Inversions *d* and *e* are always present in the ladder series of inversions which is chracteristic of certain heterozygotes; other inversions may extend the length of this ladder-like complex (Figs. 9, 10).

Inversion 2 *f* is slightly shorter than *d* and *e* and has one break point between the dark band at 2D3a and the three bands 2D3bcd. Whether this breakage point is the same as that in *e* or whether there are undetermined bands in this region that have been overlooked in the mapping is not known. The other breakage point is likewise difficult to determine, but it is proximal to the weak point at E, and is nearly at the same position as one of the breaks of *g* making the outer limit here as 2E1b. Inversion *f* always occurs between *e* and *g*.

Inversion 2*g*, one of the most common encountered, is a large rearrangement involving about a fourth of the length of the chromosome; its limits are 2E1a and 2G2a. It is present only with the included inversion *h* and in some strains *h* (2E4c and 2G1c) may be found alone. Inversion 2 *i* overlaps *h* in the presence of *g* and its outer limits are 2E3c—2F4c. Inversion 2*j* in the basal portion of the chromosome beyond *g* has breakage points at 2G2f and 2G4d.

Inversion 2*k*, a small, almost terminal inversion, which has its outer limits at 2A1b and 2A2e, may occur alone, or with *n* (2A2e—2A5g) which has only been found in the presence of *k*. Inversion 2 *l* was found only once in the study and its approximate limits are 2A2c and 2A4f.

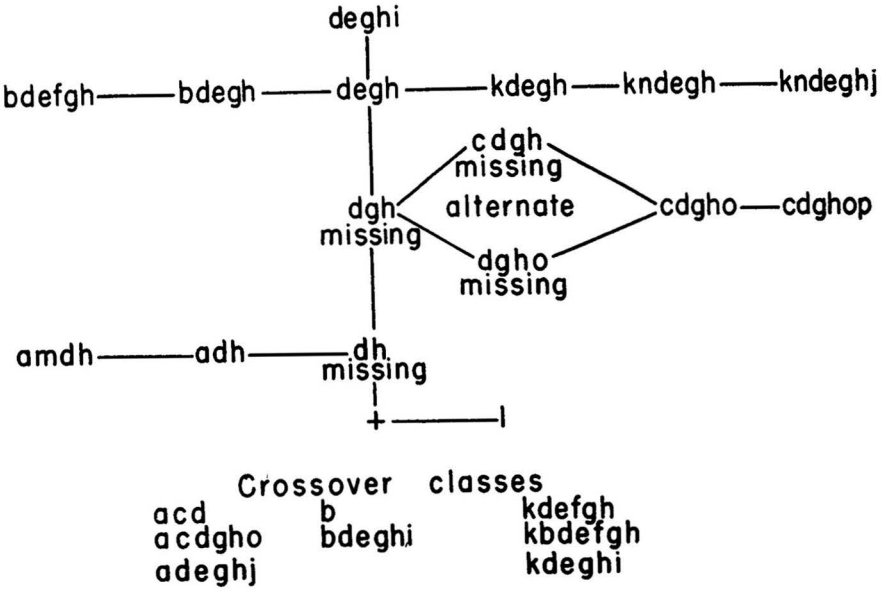


FIG. 3. Phylogeny of the gene sequences in *melancia*.

The long inversion 2 *m* (2A1b—2B6f) includes *a* and has another break point almost in common with inversion *d*, so that its limits are very difficult to determine with accuracy.

A large inversion 2 *o* overlaps *g* and probably includes *h*. These three inversions form an extremely complex configuration. Inversion *o* has its distal breakage point at 2D3d, but the other point has not yet been determined. Inversion 2 *p* (2C5h—2D2j) has been found only in association with the series of inversions *c*, *d*, *g*, *h*, and *o*.

Chromosome 3 has no inversion. Chromosome 4 has two rearrangements; 4 *a* (4E1d—4G1f) in the right arm of the chromosome is almost terminal, and 4b is longer and extends from 4C3f to 4G1d.

The chromosome variation for each strain of *melanica* tested is shown in Table 1. The comparisons are with strain 36.3c from Bastrop, Texas which has been used as the standard.

A single translocation (T XLE1d; 3E4d + 3 E4c; XLE1e) was found in checking the strain from Medicine Park, Oklahoma. All cells of the glands showed the translocation. It was not found again in checking thirty-nine other individual sibs.

DISCUSSION

Our initial problem is to determine the relationships of these gene sequences to each other. Sturtevant and Dobzhansky (1936) and Dobzhansky (1941b) have discussed the types of multiple inversions which may occur in nature and have defined three classes of such rearrangements as follows: (1) two separate inversions separated by a region in which the standard gene sequence is maintained; (2) the second inversion including the first or being included within the limits of the first inversion; and (3) the second inversion having one break outside the first inversion and the other inside (overlapping inversion). The latter is the most useful class in studying the phylogeny using rearranged gene sequences. Although it is not possible to determine by overlapping inversions which one of three gene sequences is primitive, it is possible to determine the sequence of changes with certainty.

An examination of the combinations of gene sequences of the second chromosome in the data shows that there is a total of twenty-one classes. If the distribution maps (Figs. 4, 5, 6, 7) are studied, it will be noted that the two most widespread sequences are standard and *degh* (Fig. 9). Standard ranges from Cave Creek Canyon, Chiricahua Mountains, Arizona, north to Oakdale, Nebraska, and east beyond the Mississippi and down into Florida. The *degh* sequence occupies about the same range; however it extends as far north as Poplar, Montana and as far south as Las Delicias, Chihuahua, Mexico. Both sequences extend east of the Mississippi River, but the *degh* complex does not reach Florida.

In constructing a phylogeny one should begin with the two most common sequences and attempt to fix the sequences of the occurrence of the four inversions necessary for one arrangement to give rise to the other. It is

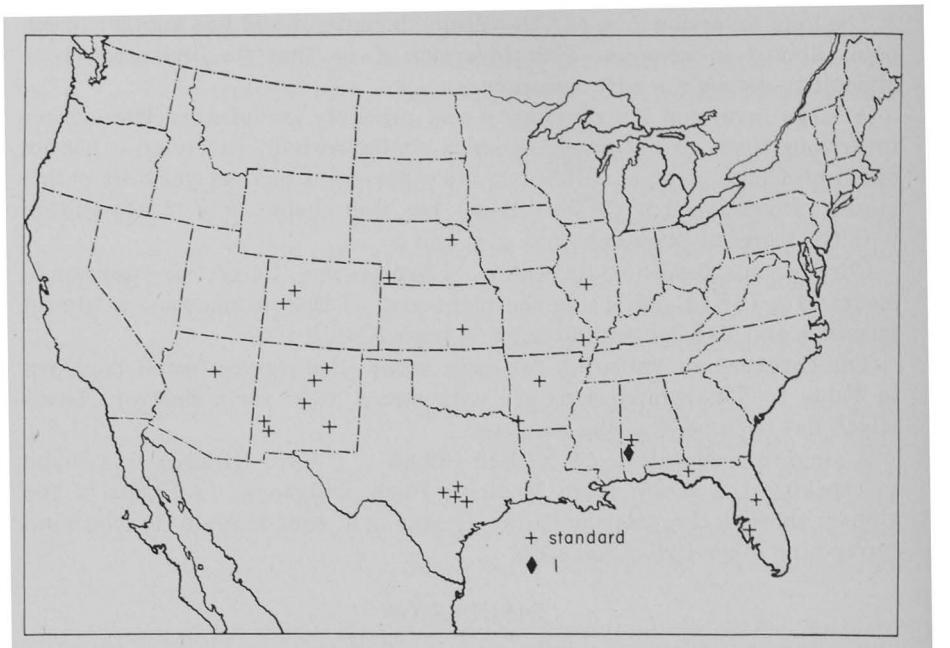


FIG. 4. Distribution of the Standard and inversion *l* in chromosome 2.

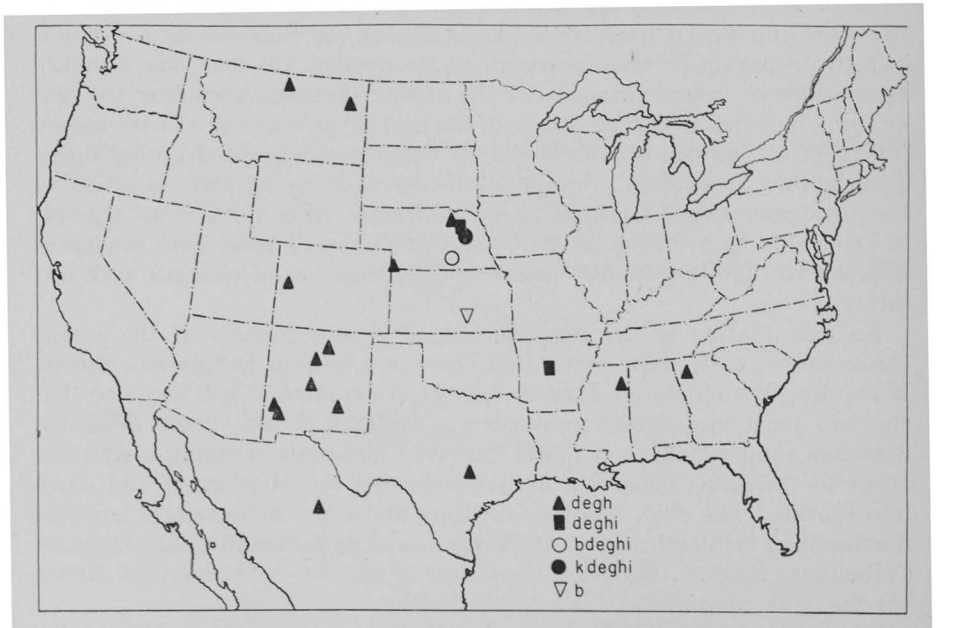


FIG. 5. Distribution of five sequences in chromosome 2.

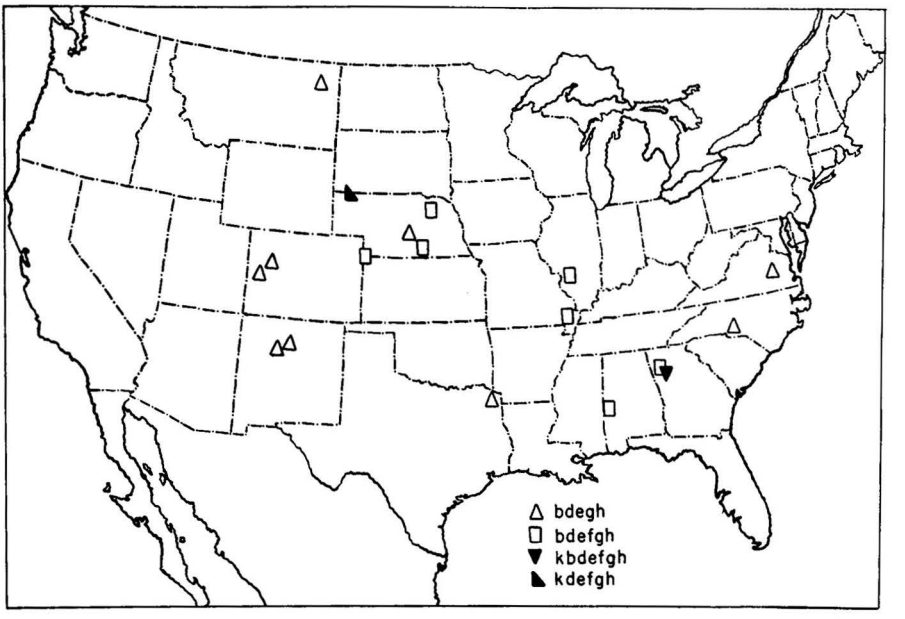


FIG. 6. Distribution of four sequences in chromosome 2.

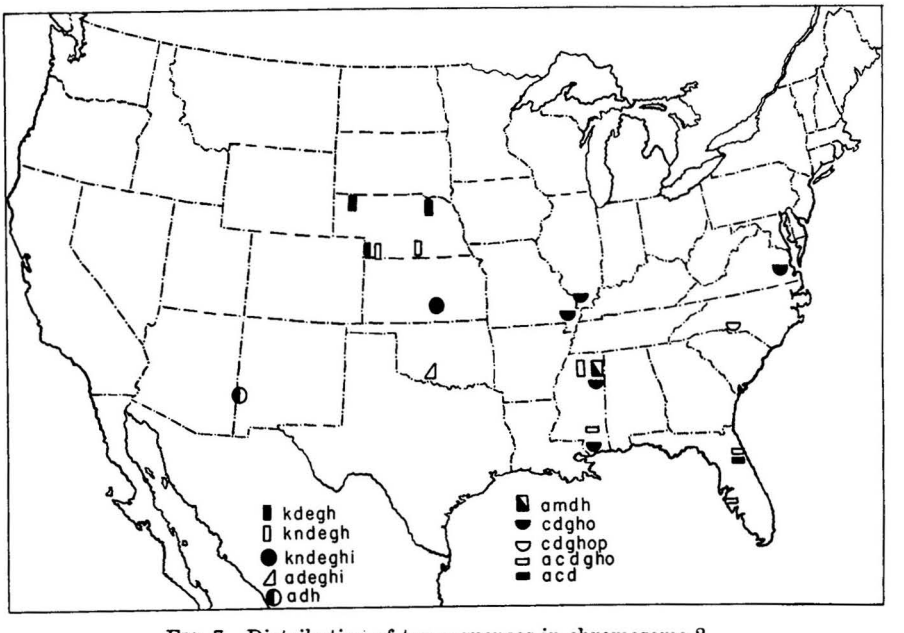
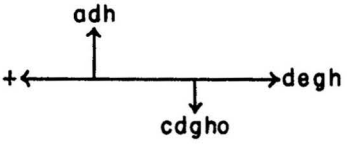


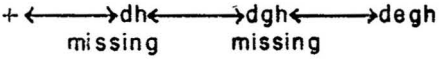
FIG. 7. Distribution of ten sequences in chromosome 2.

impossible to do this if some of the classes do not show intermediate sequences. In determining this entire phylogeny it is assumed that the inversions occur only once in the history of the species.

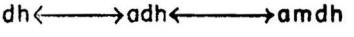
There are twenty-four possible sequences in which the arrangements could have occurred, and therefore it is necessary to look for intermediate stages. Five classes are found which show only a part of the *degh* sequences these are, *adh, amdh, cdgho, acdgho* and *cdghop* (Figs. 12, 13, 14, 15). Although these are not the intermediate steps themselves, they are derivatives of the intermediates and give some evidence as to the sequence of origin. From this information the following diagram can be drawn.



Thus it may be concluded that two of the three intermediate steps are *dh* and *dgh*, but from the data it is not possible to determine in which order *d* and *h* arose.

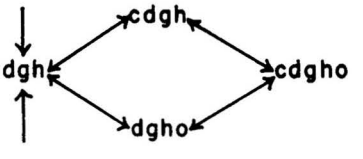


Of the classes possessing *dh* the relationship is as follows since it would be practically impossible for *m* to separate from either *a* or *d* by crossing over.



By examining the distribution (Fig. 7) it is seen that each of the sequences, *adh* and *amdh*, was found in only a single location.

The next gene sequences to be considered are the classes *cdgho, crghop, acdgho,* and *acd.* Since inversion *o* overlaps *g* and probably includes *h*, and inversion *c* includes *d*, there are possible two alternate ways of deriving the sequence *cdgho* from *dgh.* These possibilities may be diagrammed as follows:



Of these four classes only the last has been found, but the *dgh* class and either *cdgh* or *dgho* must necessarily have occurred. However, it is impossible to fix the order in which *c* and *o* occurred until an intermediate class is found. Since the inversion *p* has both its breakage points so close to those of *c* and *o*, it would be impossible for it to be eliminated from the sequence by crossing over, and therefore it must come at the end of the series after *cdgho*.

The classes *acdgho* and *acd* are interpreted to be crossover classes. This would seem logical since the class *adh* has already been found, and an individual which was heterozygous for this sequence and *cdgho* could have given rise to the class *acdgho* by crossing over between *a* and *c*, even though this is a rather short region. The reciprocal class has, however, not yet been found. The class *acd* would be due to crossing over between *acd* and *gho* when the *acdgho* sequence was heterozygous with standard.

The distribution of the sequence *cdgho* and its derivatives, *acdgho, acd* and *cdghop*, is each of the Mississippi, except in one instance where a strain from Dexter, Missouri, had the *cdgho* sequence of genes. Both *acd* and *cdghop* were found at single localities.

Inversion *l* which has been found in one strain collected in Alabama, was present in the otherwise standard gene sequence and must then have been derived from that sequence.

If one observes the chromosome map it will be seen that inversion *a* excludes the possibility of having *k*, *n*, or *b*, and that the presence of *b* excludes the possibility of having *c*. However, *b* does not exclude *k*, and *k* does not exclude *n;* in fact *n* occurs only with *k*. Since *b* excludes *k* and *n*, it would seem probable that the following sequence would hold true.

To one end of this sequence should be added *f* which cannot separate from *e* and *g*, since its breakage points are too close to those of the inversions on either side of it. If this is done, it will be necessary to class the sequences *kbdefgh* and *kdefgh* as crossover classes which could have occurred in an individual heterozygous for *bdefgh* (Fig. 10) and *kdegh*. Sequence *kdegh* would be intermediate between *degh* and *kndegh* (Fig. 11), because *k* and *n* are so close together it would be difficult to separate them by crossing over, and inversion *n* has always been found in the presence of *k*, although *k* is occasionally found alone.

Inversion *j* was found only in the strains from Wichita, Kansas and Medicine Park, Oklahoma and occurred with *kndegh* or *adegh*. Since the presence of *a* with *degh* is already considered to be the result of crossing

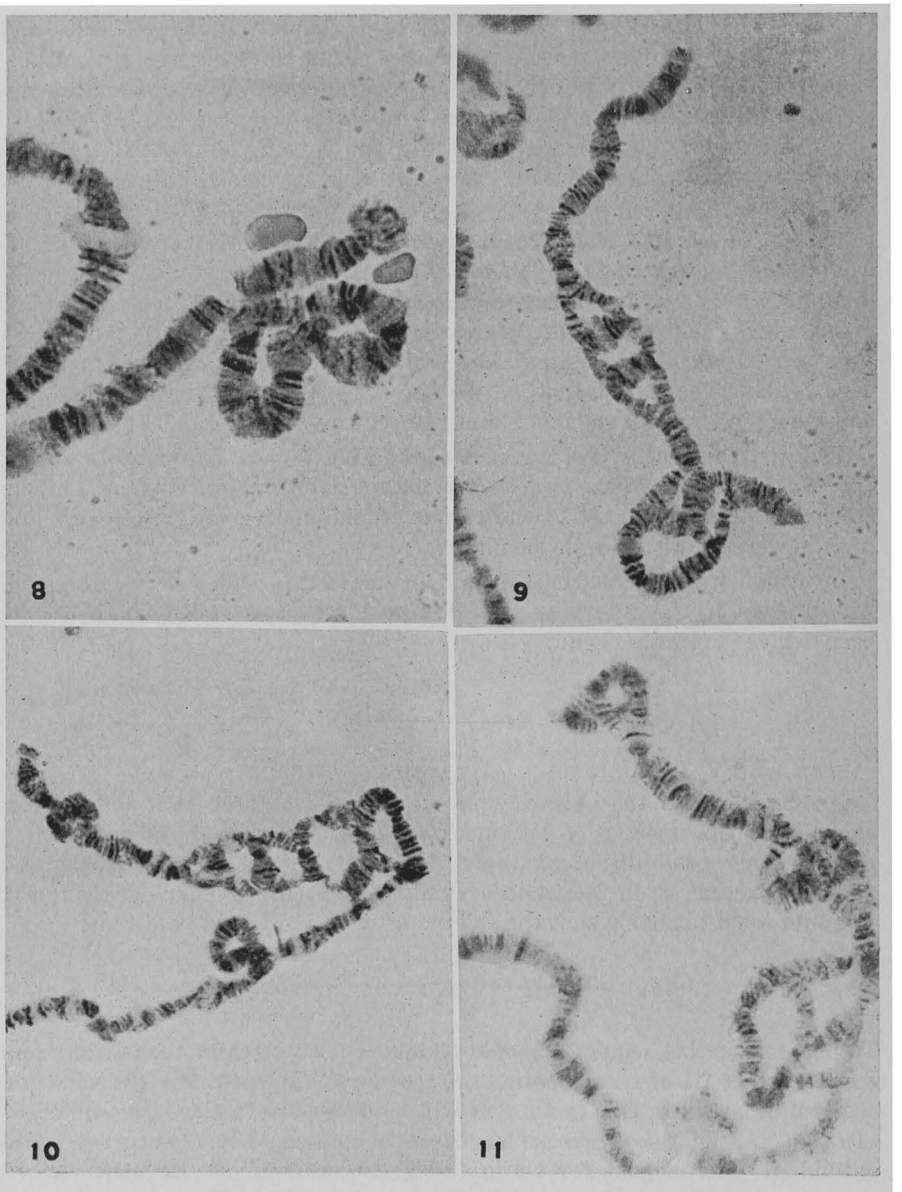


FIG. 8. Inversions *a* and *b* in the right arm of the X chromosome.

FIGS. 9 through 15 are photomicrographs of inversions in chromosome 2.

FIG. 9. Inversions *d* and *e* present at the mid-portion, and *g* and *h* in the basal part.

FIG. 10. Inversion b (left) and the ladder-like series reading from mid-region to base is *d*, *e*, *f*, *g*, and *h*.

FIG. 11. Inversions *k* and *n* at the free end, *d* and *e* in the mid-region, and *g* and *h* at the base.

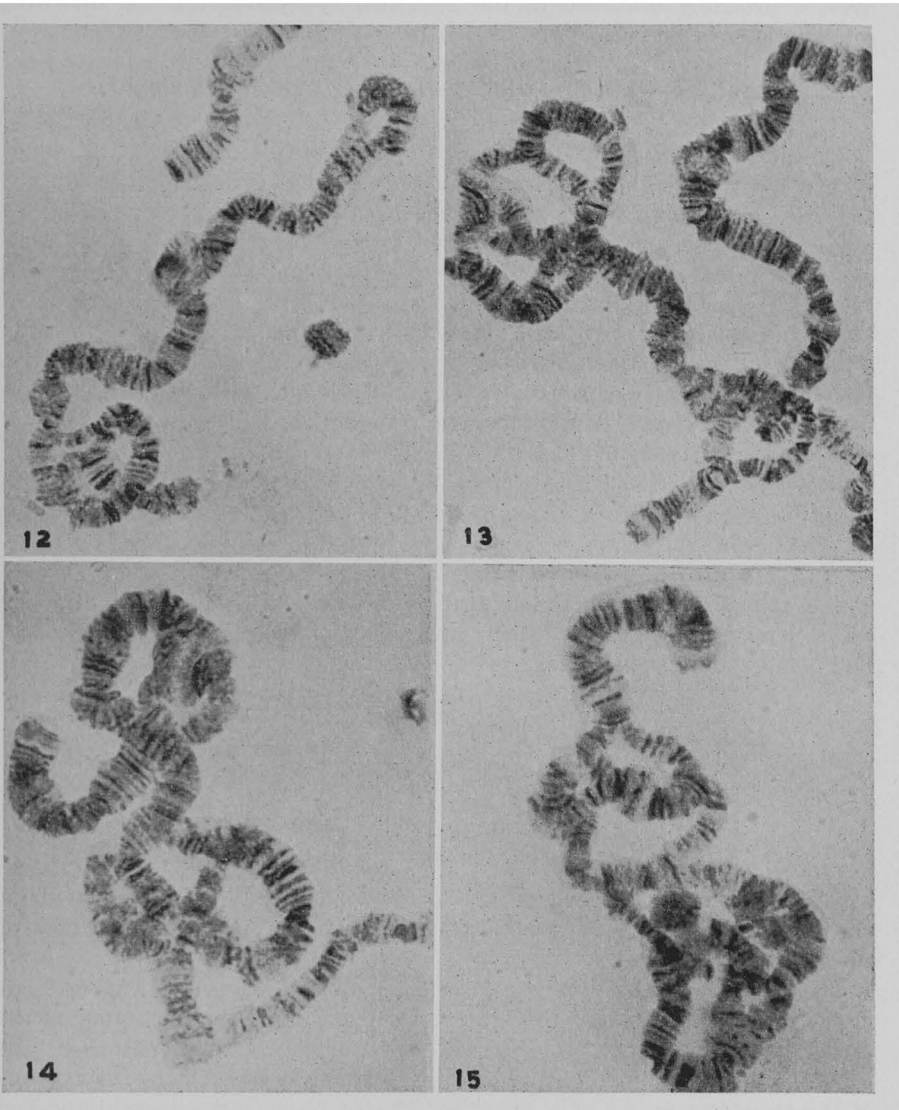


FIG. 12. Inversions *a* at upper-right and *d* and *h* at lower left.

FIG. 13. Inversions *a*, *m* and *d* forming a complex configuration at left, and inversion *h* to the right.

FIG. 14. Inversion *c* and its included inversion *d* at top, and *g*, *h*, and *o* below.

FIG. 15. Inversion *c* and *d* above and *g*, *h*, and *o* below with inversion *p* at mid-region.

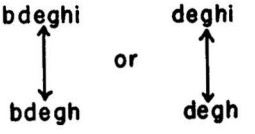
over, *j* probably occurred in the sequence *kndegh* giving the following series:

bdefgh⟷bdegh⟷degh⟷kdegh⟷kndegh⟷kndeghj

The distribution of these sequences is as would be expected with *bdegh* spread over approximately the same area as the sequence *degh*, ranging from the east coast to the westernmost limits of the species and as far north as Chinook, Montana. However, it was not found in South Texas. The distribution of *bdefgh* is not as widespread, but it occurs in Nebraska, Missouri, Illinois, Alabama, and Georgia. Each of the crossover classes, *kbdefgh* and *kdefgh*, occurs in a single location.

Sequences *kdegh* and *kndegh* occur in Nebraska except for a single strain in Mississippi which shows the latter arrangement. The sequence *kndeghj* was found in Kansas and the crossover class *adeghj* was found just south of that locality in Oklahoma.

There are now four classes left to be accounted for, the most important being the sequence *deghi* which differs from the sequence *degh* by a single inversion which overlaps *h*. Inversion *i* would necessarily have had to occur in this sequence since it overlaps *h* and is included within *g*. This leaves classes *bdeghi*, *kbdeghi*, and *b*, to be considered and these probably are crossovers. However, it is equally probable that the sequence *bdeghi* arose from *bdegh* and gave rise to *deghi*, *kbdeghi* and *b* by crossing over.



Since the distribution of the classes *b*, *deghi*, *bdeghi*, and *kbdeghi* is about the same, it would be impossible to state with the limited data which of the alternates is the more probable.

The entire phylogeny of the gene sequences is shown in Figure 3. It must be remembered that certain intermediate steps are presented as the more probable in view of the present information, but that there are other possibilities. The entire phylogeny could have arisen with any one of the sequences as the primitive one. On the basis of the distribution and the central location in the phylogeny it would seem probable that one of the two large classes of the main stem was the primitive class, either standard or *degh*. The latter is the more central arrangement of the phylogeny as the other rearrangements involve fewer changes with it as a basis.

In chromosome 4 there are two sequences which occur in addition to the standard gene sequence. Inversion *a* (Fig. 16) occurs in four locations, Chinook, Montana; Haigler, Nebraska; Medicine Park, Oklahoma; and Tombigbee Park, Mississippi, while inversion *b* (Fig. 17) occurs at all four locations in the state of Nebraska: in Wichita, Kansas; Austin, Texas;

Richmond, Virginia, and at Myakka Head and Stanford, Florida. The standard sequence occurs at every location checked and from this information it is concluded that it is probably the primitive arrangement.

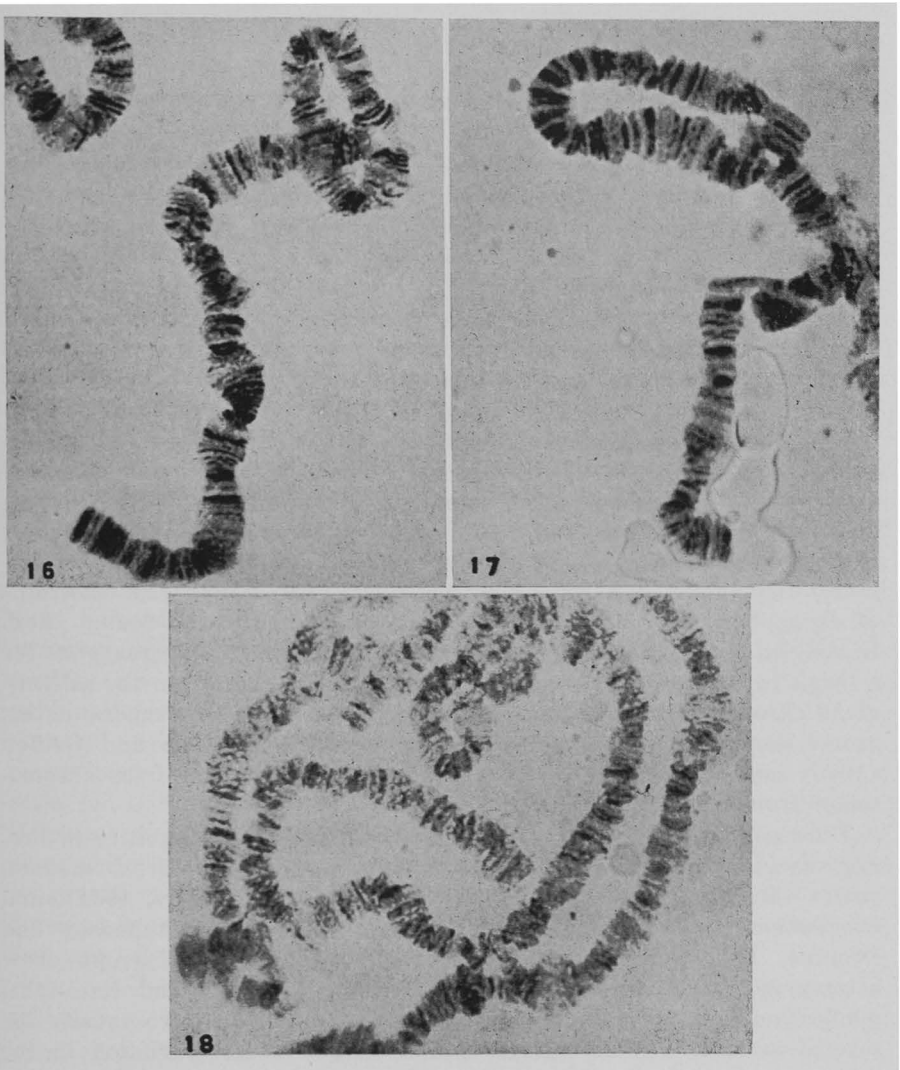
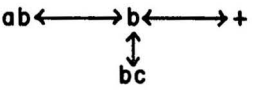


FIG. 16. Inversion *a* in chromosome 4.
FIG. 17. Inversion *b* in chromosome 4.
FIG. 18. XL-3 translocation below.

In the right arm of the X chromosome the most widespread gene sequence is *ab* (included inversion, Fig. 8). Sequences *b* and *bc* occur in Florida and Mississippi, and the sequence *b* was also found in one strain from Haigler, Nebraska. The standard gene sequence was found only in

Texas. It would seem probable that the primitive gene sequence is *ab*, and the relationships can be diagrammed as follows:



In examining the instances where cytological polymorphism has been studied in Drosophila it becomes very evident that the cases investigated thus far will fall into one of three categories: (1) species in which the gene sequence is extremely stable showing few or no inversions, and in many of these cases only a very few inversion differences exist between closely related species; (2) species where the polymorphism is about equally distributed among the various elements; and (3) the species in which the gene sequences are relatively stable with the exception of one chromosome in which there is a great deal of variation (Pavan, 1936).

The members of the repleta group which have thus far been studied cytologically fall in the first category. Wharton (1942) found no variation between strains of *repleta* from the United States, South America and Turkey, and Warters (1944) found no variation in the gene sequences of *D. meridiana* and *bifurca* and only a single inversion of almost universal distribution in chromosome 2 of *hydei*. Additional tests of strains of *D. repleta* have been reported and no variation was found. Both *D. canapalpa* and *D. melanopalpa* have been found to be heterozygous for a single inversion in the second chromosome. However, even the salivary gland chromosomes of the interspecific hybrids between members of this group show very few rearrangements. Patterson, Stone and Griffen (1940) and Warters (1944) found no variation in the *virilis* chromosomes from widely separated localities.

The second category is that which includes the species exhibiting heterozygosity in several or all of the chromosomes. Among these are *D. melanogaster* (Dubinin, Socolov, and Tiniakov, 1937, and Warters, 1944), and *D. ananassae* (Kaufmann, 1936; Kikkawa, 1938; and Dobzhansky and Dreyfus, 1943). The remaining members of the virilis group show heterozygosity in nature according to Warters (1944) and Hsu (this publication). In 1939 Dobzhansky and Socolov found heterozygosity for several inversions in *D. azteca*. Miller (1939) found variation in the gene sequence of all chromosomes of *D. algonquin* except the microchromosome. The nineteen gene sequences which Carson and Stalker (1947) reported for *D. robusta* were about evenly distributed among the chromosomes. *Drosophila willistoni* is the most variable of the species thus far studied, showing forty different inversions about evenly distributed among the chromosomes (Da Cunha, Burla, and Dobzhansky, 1950). Cavalcanti (1948) found several rearrangements in *D. prosaltans* in three of the arms of the salivary gland chromosomes.

In the third category, which includes those forms showing a concentration of inversions in a single arm, are *D. pseudoobscura* where fifteen gene arrangements have been found in the third chromosome and *D. persimilis* in which seven sequences are known. One of these rearrangements is common to the two species (Dobzhansky, 1944). In *D. nebulosa* Pavan (1946) found the third chromosome to be most variable, having seventeen gene arrangements which arose from the recombination of eight different inversions. Novitski (1946) found a disproportionately large number of inversions in element C of *D. athabasca*. *Drosophila melanica* must be placed in this group for it exhibited twenty gene sequences of chromosome 2 in addition to standard. These are the results of the combination and recombination of sixteen inversions. The right arm of the X chromosome exhibited three inversions, the left, one inversion; none were found in chromosome 3 and two were found in 4.

Using *D. melanogaster*, Bauer, Demerec and Kaufmann (1938) found that the chromosome breaks induced by X-rays were distributed more or less at random for chromosomes of comparable length, at least in the euchromatic portion. The same was found to be true by Helfer (1941) when working on X-ray induced breaks in *D. pseudoobscura*. The significance of the concentration of chromosomal rearrangements in a single chromosome of wild populations of some species of Drosophila is not known.

Dobzhansky and Epling (1948) demonstrated that heterozygosity for third chromosome inversions in *D. pseudoobscura* greatly reduced the recombination of the genes in that chromosome. This reduction in recombination serves to prevent the breakdown of certain superior gene combinations which, when present heterozygous, produce heterosis. Dobzhansky (1947 and 1948) has shown that in both artificial and natural populations the frequency of eggs deposited fits the Hardy-Weinberg ratio, but there is a differential mortality which favors the heterozygotes. Dobzhansky (1950) demonstrated that, although the heterozygotes may be superior to homozygotes when carrying gene sequences of the same geographic origin, the inversion heterozygotes carrying sequences of different geographic origin are not superior. In fact, in certain instances they are inferior to the homozygotes.

The chromosomal variability of *melanica* in relation to the color is interesting. The desert form of *D. melanica*, a very light colored fly, ranges from Chihuahua, Mexico, straight northward through New Mexico, Colorado, and up into Montana. A single western strain of the very dark form of *melanica* has been collected at Cave Creek Canyon, Arizona. It was found that the strains from Nebraska and Kansas are predominantly dark forms, but flies which show an intermediate coloration occur in these stocks. Throughout the region of the distribution of the yellow forms, the standard, *degh* and *bdegh* gene sequences are found. The Cave Creek Canyon strain was homozygous for the standard sequence, and the strains from Nebraska and Kansas, which give the variability in

color, show a great deal of heterozygosity for different gene sequences. In these states there accur twelve of the twenty-one described sequences, four of which are designated as crossover classes. In the desert strains there were found a total of four gene sequences, one of these being the rare class *adh* which was found only at Cliff, New Mexico. In the states other than Nebraska and Kansas, which have only the dark form of *melanica,* there occur fourteen of the gene sequences.

Spontaneous translocations are of interest to us because they are extremely rare. Dobzhansky and Dreyfus (1943) found that two out of seven females of *D. ananassae* collected at Mogi das Cruzes, Brazil, were either translocation heterozygotes themselves or had mated with a male carrying a translocation. Cavalcanti (1948) reported a translocation or whole arm exchange in *D. prosaltans.* The translocation between XL and 3 of *D. melanica* (Fig. 18) probably occurred in the laboratory since the strain was maintained in culture for several years before this study was made.

SUMMARY

1. In examining sixty-two strains of *D. melanica melanica* Sturtevant, a total of twenty-two inversions was found, three in the right arm of the X chromosome, one in the left arm, sixteen in chromosome 2, none in 3 and two in chromosome 4. The sixteen inversions in the chromosome 2 give twenty gene sequences, in addition to the standard arrangement. Thus *melanica* may be classed among the species which exhibit a great variability of gene sequences in a single chromosome.

2. By using overlapping and very closely linked inversions a phylogeny of gene sequences has been formulated. It is not possible to determine the primitive gene sequence, but on the basis of the distribution and derivative classes, either *degh* or standard is most probably that class.

3. The desert form of *melanica* was found to have four different gene sequences. The neighboring populations in the states of Nebraska and Kansas, which exhibit color variation, had twelve different gene sequences, and in the remaining states fourteen of the twenty-one gene sequences were found.

4. A single individual heterozygous for a translocation was found in the strain from Medicine Park, Oklahoma.

REFERENCES

Bauer, H., M. Demerec, and B. P. Kaufmann. 1938. X-ray induced chromosomal alterations in *Drosophila melanogaster.* Genetics, 23:610–630.

Carson, H. L. and H. D. Stalker. 1947. Gene arrangements in natural populations of *Drosophila robusta* Sturtevant. Evolution, 1:113–133.

Cavalcanti, A. G. L. 1948. Geographic variation of chromosome structure in *Drosophila prosaltans.* Genetics, 33:529–536.

Cunha, A. B. da, H. Burla, and Th. Dobzhansky. 1950. Adaptive chromosomal polymorphism in *Drosophila willistoni.* Evolution, 4:212–235.

Dobzhansky, Th. 1941a. Discovery of a predicted gene arrangement in *Drosophila azteca.* Proc. Nat. Acad. Sci., 27:47–50.

Dobzhansky, Th. 1941b. Genetics and the Origin of Species. Second Edition. Columbia Univ. Press.

Dobzhansky, Th. 1947. Genetics of natural populations. XIV. A response of certain gene arrangements in the third chromosome of *Drosophila pseudoobscura* to natural selection. Genetics, 32:142–160.

Dobzhansky, Th. 1948. Genetics of natural populations XVII. Proof of operation of natural selection in wild populations of *Drosophila pseudoobscura*. Genetics, 33:537–547.

Dobzhansky, Th. 1950. Genetics of natural populations XIX. Origin of heterosis through natural selection in populations of *Drosophila pseudoobscura*. Genetics, 35:288–302.

Dobzhansky, Th., H. Burla, and A. B. da Cunha. 1950. A comparative study of chromosomal polymorphism in sibling species of the willistoni group of Drosophila. Amer. Nat., 84:229–246.

Dobzhansky, Th. and A. Dreyfus. 1943. Chromosomal aberrations in Brazilian *Drosophila ananassae*. Proc. Nat. Acad. Sci., 29:301–305.

Dobzhansky, Th. and C. Epling. 1944. Contributions to the genetics, taxonomy, and ecology of *Drosophila pseudoobscura* and its relatives. Carne. Inst. Wash. Publ. 554.

Dobzhansky, Th. and C. Epling. 1948. The suppression of crossing over in inversion heterozygotes of *Drosophila pseudoobscura*. Proc. Nat. Acad. Sci., 34:137–141.

Dobzhansky, Th. and D. Socolov. 1939. Structure and variation of the chromosomes of *Drosophila azteca*. Jour. of Hered., 30:3–19.

Dubinin, N. P., M. N. Sokolov, and G. G. Tiniakov. 1936. Occurrence and distribution of chromosomal aberrations in nature. Nature, 137:1035–1036.

Dubinin, N. P., M. N. Sokolov, and G. G. Tiniakov. 1937. Intraspecific chromosome variability. Jour. of Biol. (Russian), 6:1007–1054.

Griffen, A. B. 1942. Relationships in the melanica species group. Univ. of Tex. Publ. 4228:68–73.

Helfer, R. G. 1941. A comparison of X-ray induced and naturally occurring chromosomal variations in *Drosophila pseudoobscura*. Genetics, 26:1–22.

Hsu, T. C. Chromosomal variation and evolution in the virilis group of Drosophila. (this publication).

Hughes, R. D. 1939. An analysis of the chromosomes of the two subspecies *Drosophila virilis virilis* and *Drosophila virilis americana*. Genetics, 24:811–834.

Kaufmann, B. P. 1936. A terminal inversion in *Drosophila ananassae*. Proc. Nat. Acad. Sci., 22:591–594.

Kikkawa, H. 1938. Studies of the genetics and cytology of *Drosophila ananassae*. Genetica, 20:458–516.

Miller, D. D. 1939. Structure and variation of the chromosomes in *Drosophila algonquin*. Genetics, 24:699–708.

Novitski, E. 1946. Chromosome variation in *Drosophila athabasca*. Genetics, 31:508–524.

Painter, T. S. 1933. A new method for the study of chromosome rearrangements and the plotting of chromosome maps. Science, 78:585–586.

Patterson, J. T., W. S. Stone and A. B. Griffen. 1940. Evolution of the virilis group of Drosophila. Univ. of Tex. Publ., 4032:218–250.

Patterson, J. T., W. S. Stone and A. B. Griffen. 1942. Genetic and cytological analysis of the virilis species group. Univ. of Tex. Publ., 4228:162–200.

Pavan, C. 1946. Chromosomal variation in *Drosophila nebulosa*. Genetics, 31:546–557.

Sturtevant, A. H. 1931. Known and probable inverted section of the autosomes of *D. melanogaster*. Carne. Inst. Wash. Publ., 421:1–27.

Sturtevant, A. H. and Th. Dobzhansky. 1936. Inversions in the third chromosome of wild races of *Drosophila pseudoobscura*, and their use in the study of the history of the species. Proc. Nat. Acad. of Sci., 22:448–450.

Warters, M. 1944. Chromosomal aberrations in wild populations of Drosophila. Univ. of Tex. Publ., 4445:129–174.

Wharton, L. T. 1942. Analysis of the repleta group of Drosophila. Univ. of Tex. Publ., 4228:23–42.

X. *DROSOPHILA EURONOTUS*, A NEW MEMBER OF THE MELANICA SPECIES GROUP

J. T. PATTERSON AND C. L. WARD

INTRODUCTION

In connection with a study of chromosome variation in *Drosophila melanica* Sturtevant, the junior author found that certain strains of what were supposed to represent this species failed to cross to the stock used as the standard. The strains in question originated from flies collected in the southeastern part of the United States. Stocks of some of these strains had been in the laboratory for as long as three years; others had been established from collections made more recently. On account of its darker color, the new form had been observed in collections made as early as 1941, but was regarded merely as a color phase of the typical *melanica*. It was not until after the cross-tests had been carried out that its rank as a different species became evident.

The strains upon which the following description and genetic tests were made are as follows:

Two strains from Krotz Springs, Louisiana (lot #2002.7)
Fourteen strains from Hollandale, Mississippi (lot #2021.5)
Four strains from Tombigbee State Park, Mississippi (lot #2020.7)
Six strains from Tallahassee, Florida (lot #2007.7)
One strain from Golden Head Branch State Park, Florida (lot #2012.7)
One strain from Indian Springs, Georgia (lot #2015.7)
One strain from McRae, Georgia (lot #1876.4)
Four strains from Twin Lakes, Georgia (lot #2014.4)
One strain from Williamston, North Carolina (lot #1876.3)

Of the thirty-four stocks used in the study, each of twenty-three were developed from a single fertile female taken in nature.

Drosophila euronotus, sp. nov.

External characters of imagines.

♂. Arista with about 8 branches, 2 below in addition to the fork; the smaller hairs along the axis, at right angles to the usual branches, longer than usual, about as long as branches of terminal fork. Front light brown with slight reddish cast, orbits and ocellar triangle darker brown, granulose. Proclinate orbital about ¾ length posterior reclinate, anterior reclinate thin, a little over ⅓ length proclinate. Antennae brown, 3rd joint a little darker. Face pale brown, darker on vibrissal angles. Carina broad and flat, very shallowly sulcate. One strong oral bristle, the 2nd thin, about half length first; posterior angle of cheek with about 3 stronger bristles. Palpi grayish yellow, flattened, with several prominent hairs. Cheeks gray, narrow, their width about ⅛ greatest eye diameter. Eyes dark red with long, black pile.

Acrostichals clearly in 6 rows; no prescutellars. Anterior scutellars strongly convergent. Mesonotum and scutellum dark blackish brown, quite dull, with indistinct lighter pollinose markings on humerus, along transverse suture, in alar region and on basal angles of scutellum. Pleurae, especially sternopleurae, darker than disc of mesonotum, more heavily pollinose. Sterno-index about 0.8. Halteres pale yellow. Legs brownish black, mid- and hind-femora and all tarsi paler. Apicals on 1st and 2nd tibiae, preapicals on all three.

Abdominal tergites of male with somewhat indistinct apical brownish bands, less than half the width of the tergites, widely interrupted medianly on basal segments, increasingly less so on terminal segments. The bands become indistinct on lateral areas, more so on posterior segments. Genital arch entirely pale. Abdominal bands of female darker, more distinct, and with more clearly marked interruptions than on male. On the basal five tergites the bands bend forward at the angle, forming solid lateral areas, smaller posteriorly; on the next tergite the band ceases before reaching the margin and is not connected with a small dark lateral area. The pregenital tergite is entirely yellow.

Wings uniformly dusky, veins brown, crossveins not noticeably clouded. Costal index about 3.7; 4th vein index 1.6; 5x index about 1.0; 4c index about .5. Two medium sized bristles at apex of first costal section. Third costal section with heavy bristles on its basal ⅓.

Length body 3 mm. (in live specimen); wings 2.5 mm.

♀. Length body 3.5 mm.; wings 3 mm.

Internal characters of imagines.

Testes light orange, with five inner and six outer coils or gyres. Ejaculatory sac with two anterior and two posterior diverticula.

Spermathecae sclerotized; ventral receptacle long spiral with about fifty-five fine coils.

Other characteristics, relationship and distribution.

Eggs.—2 slightly curved filaments, each about ¾ length of egg.

Puparia.—Cream colored; horn-index about 8.6; anterior spiracle with 7 branches. At the approach of pupation the larvae arrange themselves in parallel groups on the inner surface of container, forming raft-like figures.

Chromosomes.—Metaphase plate shows two pairs of rods, a pair of small V's, a pair of large V's, the X chromosomes, and a pair of dots. The Y chromosome is J-shaped. The salivary gland nuclei have six long strands and a dot.

Relationship.—Belongs to the melanica group of the subgenus Drosophila. Its closest relative is *D. melanica paramelanica.*

Distribution.—Its distribution range occurs in the southeastern corner of the United States, where it has been identified at nine different localities (see Fig. 1).

Types.—Type material was derived from a single fertile female collected at Krotz Springs, Louisiana. Holotype and nine paratypes (No. 2002.7), descendants of the original female, placed in the collection of The University of Texas.

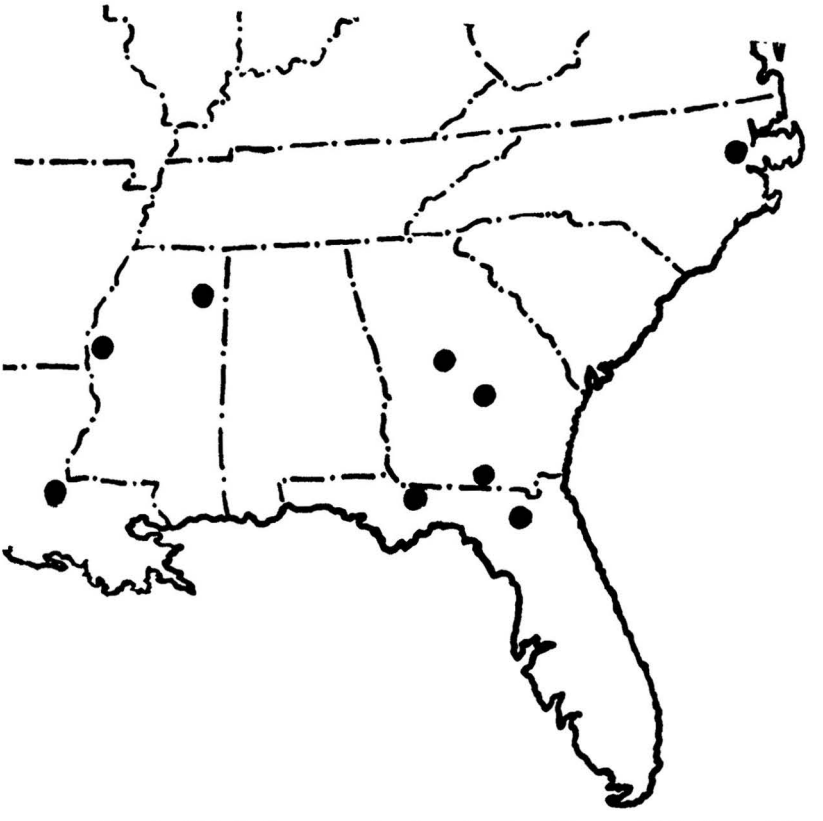


FIG. 1. Distribution map showing nine localities at which *Drosophila euronotus* has been identified.

GENETIC TESTS

Crosses between *D. euronotus* and *D. melanura* and the two subspecies, *D. melanica melanica* and *D. melanica paramelanica* were carried out. The stocks employed and their origins used in these tests were as follows:

euronotus, Stock 2002.7b from Krotz, Louisiana
melanura (like), Stock 1759.3 from Devil's Lake, N. Dakota
melanica, Stock 36.3c from Bastrop, Texas
paramelanica, Stock 1891.6 from Guarette, Maine

The matings were made in large vials, each containing ten pairs of flies which had been aged from five to eight days. The flies were transferred once to fresh food and held from four to five weeks. In the P_1 matings the

reciprocal crosses of *euronotus* to *melanica* and *melanura*, as well as the *paramelanica-euronotus* cross, were all incompatible (Table 1, 1–5). But in the *euronotus-paramelanica* cross (6), 27 of the 63 mass matings yielded 225 offspring for an average of about 0.35 offspring per tested pairs, indicating the presence of a large amount of sexual isolation.

TABLE 1

Shows results from crosses between *euronotus* and *melanica*, *melanura* and *paramelanica*

Crosses ♀ ♂	Number tested	Number of offspring	Females	Males
P_1 matings				
1. melanica × euronotus	580	0	.....	.....
2. euronotus × melanica	700	0	.....	.....
3. melanura × euronotus	60	0	.....	.....
4. euronotus × melanura	40	0	.....	.....
5. paramelanica × euronotus	710	0	.....	.....
6. euronotus × paramelanica	630	225	115	110
Inbred and backcrosses				
7. $F_1 \times F_1$	27♀ + 16♂	0	.....	.....
8. euronotus × F_1	10♂ ♂	0	.....	.....
9. paramelanica × F_1	19♂ ♂	0	.....	.....
10. F_1 × euronotus	15♀ ♀	41	18	23
11. F_1 × paramelanica	29♀ ♀	745	389	356

The *euronotus/paramelanica* hybrids were as light in color as *paramelanica* and very sensitive to ether. They were inbred, and also backcrossed to their parental forms (Table 1, 7–11). In the inbred test 27 females and 16 males were mated together, but failed to produce offspring. The backcrosses of the F_1 males to *euronotus* and *paramelanica* females both failed to produce offspring (8, 9), obviously due to the sterility of the males, as dissections showed that their testes did not contain mature sperm. The backcrosses of the F_1 females to *euronotus* and *paramelanica* males were both fertile, giving 41 and 745 offspring, respectively. Thus the first cross was much less fertile than the second, producing less than three offspring per tested female. The F_2 flies (1st backcross generation) were inbred, but the cross to *euronotus* failed to produce progeny, due, in all probability, to the development of mold in the culture. The flies from the cross to *paramelanica* went very well in inbred both in the first and second backcross generations.

The conclusion to be drawn from the results from these cross-tests is that the closest relative of *euronotus* in the group is the subspecies *paramelancia.*

XI. THE DROSOPHILIDAE OF THE NEARCTIC REGION, EXCLUSIVE OF THE GENUS DROSOPHILA

MARSHALL R. WHEELER

INTRODUCTION

In the United States the Drosophilidae comprise the third largest family of acalyptrate Diptera, exceeded in the number of species only by the Ephydridae and Chloropidae. The present account is an attempt to summarize the Nearctic genera and species of the family, exclusive of those of the genus *Drosophila,* in such a manner as to make their identification possible by the non-specialist. Many changes have been made in the systematics of this family since the last major revision (Sturtevant, 1921), and it is believed that a summary at this time may be of considerable benefit to the many students of evolution who deal primarily with the species of *Drosophila.* At the present time investigators in this field are placing increasing importance on natural populations, ecological relationships, food habits, and related fields. In many of these studies species of other genera are often encountered and may well be of importance in our understanding of population competition.

In the hope of making identification of the relatives of *Drosophila* easier, entirely new keys to the genera and species have been prepared from actual specimens and from stocks maintained by the Texas laboratory and from additional material borrowed from other interested persons. The terminology has been kept as non-technical as possible but some knowledge of the Diptera, especially *Drosophila,* is expected.

Following the generic key, the respective genera are discussed in alphabetical order, with keys to the known North American species as well as additional information concerning their distribution, natural history, internal morphology and some comments on methods of rearing.

The area covered by this summary corresponds most nearly to the continental United States, although species from northern Mexico and from Canada are included. A few extra-limital species have been included when specimens were available for adequate study but for most Central American species the papers of Duda (1925, Costa Rica; 1927, South America) and Malloch (1924a, 1926, Costa Rica, Panama) will be of value.

Members of the genus *Drosophila* have been excluded for several reasons. Keys to the North American species have been published rather recently (Sturtevant, 1942; Patterson, 1943) and, although there are imperfections in these keys and they fail to include more recently described species, they are fairly adequate for the present. Further, the genus is so large that a detailed summary comparable to the present one is an extremely imposing task, one which the writer hopes to accomplish within the coming year.

Many of the observations recorded here were made while the author held a Gosney Fellowship at the California Institute of Technology and he

wishes to express his gratitude for the honor. Dr. A. H. Sturtevant has been especially helpful in lending his extensive collection of Drosophilidae for study and has added considerably to the following keys by his suggestions. Other workers, too numerous to mention, have also contributed specimens of various genera for our use.

The type specimens of the thirteen new species described in the present paper have been deposited in the Drosophila Type and Reference Collection of The University of Texas, Austin, except in the few cases which are noted in the text. In this treatment of the family 20 genera are reported from the area and 45 named species are recognized, not including 13 which are described as new. In addition, 14 species are included without formal naming, thus giving a total of 72 species, exclusive of the genus *Drosophila.*

DIAGNOSIS OF THE FAMILY

At the time of Sturtevant's 1921 monograph this group was treated as a subfamily, the Drosophilinae, of the family Muscidae. Since then, the various subfamilies have generally been accorded family rank and many of them have, in fact, been divided into several families. As a consequence it seems wise to indicate now the limits of the family Drosophilidae as interpreted by the writer and a majority of present-day Dipterists. The family may be defined as follows:

Acalyptrate Diptera with bare mesopleura, twice-broken costa, rudimentary subcosta (auxiliary vein), typically with 3 orbitals, the anterior pair proclinate, the others reclinate, and with the post-vertical bristles (postocellars) convergent though sometimes quite small. Sternopleurals are typically present, the vibrissae are usually developed, the proclinate orbital is not nearer the eye margin than the anterior reclinate, and the disc of the scutellum is bare although in a few species there are a few marginal hairs in addition to the usual four scutellars. The arista may be bare, pubescent or plumose, the 2nd basal and discal cells of the wing may be confluent or separated, the anal vein may be present or absent, and the costal margin is not usually spinulose. In many species the eyes are bright red in life, and the eggs often possess anterior filaments. The size range is quite extreme, from less than 1 mm. to 7 mm. or perhaps larger.

It is to be noted that this diagnosis excludes from the family certain genera often included within it. One of the more recent works dealing with the family as a whole is that of Seguy (1934) who recognizes three subfamiles as follows: **Curtonotinae**, containing only *Curtonotum;* **Diastatinae,** with *Diastata, Tryptochaeta* and *Euthychaeta;* and **Drosophilinae,** with all the usual drosophiloid genera but also including the aberrant *Cryptochaetum.* All of the genera of the first two groups, except *Tryptochaeta,* have bristled mesopleurae and are often segregated into a single family. *Tryptochaeta,* belonging with this group, has the proclinate orbital placed nearer the eye margin than is the anterior reclinate, and also differs in several internal characters from the usual *Drosophila* pattern.

Cryptochaetum is a genus of parasitic flies with a number of aberrant characters and has been placed in a variety of families by different writers. The present trend is to place it in a family of its own, the Cryptochaetidae. More recently, Seguy (1951) has adopted the general conclusions reached above,listing separately the families Drosophilidae, Diastatidae and Cyrtonotidae, but includes, however, *Cryptochaetum* in the Drosophilidae.

A more restrictive arrangement of the family is given by Brues and Melander (1945) who divide the Drosophilidae into four subfamilies as follows: **Camillinae**, eg., *Camilla;* **Steganinae**, eg., *Stegana;* **Amiotinae**, eg., *Amiota, Sinophthalmus, Orthostegana;* and **Drosophilinae**, with a number of miscellaneous genera. This arrangement, while limiting the family more nearly in accord with the writer's viewpoint, still includes *Camilla* whose bristly mesopleura, lack of sternopleurals and other unique characters have led other workers to segregate it into a family Camillidae.

In the writer's opinion, attempts to establish subfamilies seem rather premature at present. The **Amiotinae** would seem to be a valid group and is probably the most primitive group in the family (cf. Sturtevant, 1942: 26), containing, of our genera, *Amiota, Cacoxenus, Sinophthalmus, Leucophenga, Rhinoleucophenga, Gitona* and perhaps *Stegana* as an offshoot. Within the **Drosophilinae** several evolutionary lines seem indicated. The subgenus *Hirtodrosophila* of *Drosophila* seems clearly related to *Zygothrica* on the one hand and to *Scaptomyza* on the other hand, the latter in turn showing relationship to *Chymomyza* and *Bunostoma,* and with *Neotanygastrella* related to these two. *Mycodrosophila* seems obviously related to *Dettopsomyia* and the oriental *Spuriostyloptera.* However, the majority of the genera do not show clear relationship with any others, owing, in large part, to the lack of essential information concerning them.

KEY TO THE NEARCTIC GENERA OF DROSOPHILIDAE

1. Arista plumose, with several dorsal branches and usually one or more ventral branches basal to the terminal fork 7
   Arista bare, pubescent, or with a single long dorsal branch near base with the main axis pubescent and not bifurcate apically 2
2. Arista with one long dorsal branch basally; no prescutellars; face rather flat; wings dusky, especially along costal edge, the crossveins clouded ..... **CLADOCHAETA** p. 180
   Arista bare or pubescent along its length; prescutellars present; other characters not entirely as above 3
3. No proclinate orbital; face carinate between antennae and noticeably protuberant below, with shining white pruinose areas; shining black species with middle and hind knees and tarsi yellow; size 1.8 mm. ..... **CINDERELLA** p. 180
   Proclinate orbitals present; otherwise not as above 4
4. Face distinctly carinate; mesonotum with spotted pattern 6
   Face flat, not carinate, or with at most a low median ridge which does not even simulate a nose-like carina; mesonotum unicolorous or with faint spotted appearance 5
5. Anterior frontal bristles rather convergent; propleurals absent; mesonotum reddish brown, without spots; wings with dark pattern in cells and on veins ..... **PSEUDIASTATA** p. 192
   Anterior orbitals clearly proclinate; propleurals absent; mesonotum dull, dark brownish black with faintly indicated spots around hair bases; wings without pattern ..... **CACOXENUS** p. 173

6. No differentiated sutural bristle just anterior to transverse suture; legs well marked with dark bands; wings with dark clouds on crossveins ..... **SINOPHTHALMUS** p. 208

   Sutural bristles well developed; legs not banded or with at most faint bands apically on femora and basally on tibiae; wings unmarked ..... **GITONA** p. 183

7. Postvertical bristles greatly reduced in size, much smaller than anterior reclinate orbital, often appearing nearly absent; propleurals usually present 8

   Postverticals well developed, usually as large as or larger than anterior reclinate orbital; propleurals present or absent 14

8. Face flat, without a true carina 9

   Face carinate, at least above 10

9. Mostly small species (1.0–2.5 mm.); costa reaching 4th vein; 3rd costal section without thorn-like warts below; propleurals absent; prescutellars present or absent ..... **DIATHONEURA** p. 182

   Mostly large species (2.5–6.5 mm.); costa reaching 3rd vein or slightly beyond; 3rd costal section usually with thorn-like warts on under side ..... **LEUCOPHENGA** p. 184

10. Anterior reclinate orbital placed anterior to proclinate and as long or longer than the latter, the proclinate pair rather convergent, sometimes strongly so; no prescutellars; acrostichal hairs in 8 rows or less ..... **CHYMOMYZA** p. 173

    Anterior reclinate orbital placed well behind proclinate and usually weaker than the latter, the proclinates not noticeably convergent; prescutellars strong; acrostichals in 8 or more rows 11

11. Middle tibiae with a series of stout black bristles on basal outer half or less; 3rd costal section with small thorn-like warts on under side; wings often strongly bent down at sides ..... **STEGANA** p. 211

    Middle tibiae without such extra basal bristles; costa with or without warts on 3rd section; wings seldom bent down at sides 12

12. Mesonotal hairs and bristles arising from spots; legs largely pale yellow with faint bands apically on femora and basally on tibiae ..... **GITONA** (part) p. 183

    Mesonotum usually unicolorous yellow, brown or black; if somewhat spotted then the legs are conspicuously banded 13

13. Front large, densely haired anterior to ocelli; discal and 2nd basal cells confluent; never with milky white areas on face and humeri ..... **RHINOLEUCOPHENGA** p. 193

    Front sparsely haired; discal and 2nd basal cell separated by a colored crossvein; many species with milky white areas on face and humeri and below wing bases ..... **AMIOTA** p. 166

14. Prescutellars strong, distinctly larger than the usual acrostichal hairs 15

    No prescutellars, the hairs in this position not enlarged 18

15. Posterior reclinate orbital much closer to inner vertical than it is to the proclinate orbital; acrostichals in 10 or more rows 16

    Posterior reclinate orbital usually obviously closer to proclinate than to inner vertical; if, rarely, it is about midway between them, then there are fewer than 10 acrostichal rows 17

16. Large vibrissae followed by a row of short, weak bristles; abdomen with distinct pattern ..... **LEUCOPHENGA** (part) p. 184

    Large vibrissae followed by a row of long, stout bristles; abdomen pale tan to brownish, without pattern ..... **GENUS X** p. 216

17. Face flat, not carinate; one strong sternopleural; anal vein and cell practically absent; in our species with wings heavily clouded, especially along anterior half ..... **CLASTOPTEROMYIA** p. 180

    Face more or less obviously carinate; usually with 2–3 sternopleurals; anal vein present or not; wings various ..... **DROSOPHILA** (part)

18. Fore femora, tibiae and metatarsi dark brown to black, contrasting with the pale yellow to white apical tarsal joints; other legs pale; carina low between antennae, often bulbous below; anterior reclinate orbital small, placed slightly in front of and to the side of the proclinate ..... **NEOTANYGASTRELLA** p. 192

    Fore legs usually similar to the others in coloration; other characters not entirely as above 19

19. Anterior dorsocentrals placed far forward, nearly level with the transverse suture, the distance between them usually less than that between the anterior and posterior ones 20
Anterior dorsocentrals (excluding any extra ones) well behind suture, closer to posterior ones than to each other, or at least about equally close to them 21

20. Distal costal incision exceptionally deep, the costa greatly enlarged basal to the incision, black in color and protruding beyond wing margin; apical scutellars crossed; acrostichals in about 4 rows; front rather flat; small dark species with complex mesonotal pattern ..... **DETTOPSOMYIA** p. 182
Distal costal incision not deep nor the costa enlarged; apical scutellars widely divergent; acrostichals in about 8 rows; front about twice as broad as long, the orbits separated from the ocellar triangle by a V-shaped depression; small yellow species ..... **MICRODROSOPHILA** p. 189

21. Acrostichals in 2 or 4 rows both between dorsocentrals and in front of them; rather slender species ..... **SCAPTOMYZA** p. 194
Acrostichals in 6 or more rows at the level of the anterior dorsocentrals 22

22. Semi-shining flies with rather uniformly brownish to blackish mesonotum, scutellum and upper pleurae, the lower pleurae and under surfaces, including legs, pale whitish to yellowish, strongly contrasting with the dark upper parts; distal costal incision usually pronounced; posterior notopleural bristle farther above notopleural suture than anterior bristle ..... **MYCODROSOPHILA** p. 190
Not entirely as above; if the mesonotum is uniformly dark then the under surfaces are not contrastingly paler; posterior notopleural about as near suture as is the anterior one 23

23. Front often longer than wide, usually with a noticeably enlarged frontal triangle reaching nearly to antennal bases; cheeks usually protuding far in front of eyes, the oral margin deeply incised medianly, semi-circular and turned up toward carina; proboscis exceptionally long when extended; arista usually with a single ventral branch basal to the fork; face strongly carinate; some ♂ ♂ of *Z. dispar* with head and eyes grotesquely extended and pointed laterally ..... **ZYGOTHRICA** p. 213
Not entirely as above; front usually as wide as long or nearly so; rarely with a distinct frontal triangle extending beyond ocellar area; if the arista has but a single ventral branch then the carina is limited to upper part of face ..... **DROSOPHILA**

AMIOTA Loew

1862. Berl. ent. Zeit., 6:229.

Genotype: *A. humeralis* Loew.

? = *Phortica* Schiner, 1862. Wien. ent. Monat., 6:433.

Most authorities seem agreed that *Amiota* and *Phortica* are synonymous, and according to Malloch and McAtee (1924) Schiner's publication appeared several months after that of Loew, and hence *Amiota* should be used. European workers, however, have not accepted *Amiota* as valid and use *Phortica*. The type of the latter, *variegata* (Fallén), is distinct in many characteristics from *humeralis* and its closest relatives, and I feel that ultimately they will be separated into different genera. However, the group of species concerned is very poorly understood at present, taxonomically, so that for the present it seems best to place *Phortica* as a subgenus, as was first suggested by Sturtevant (private communication). Further, I do not feel that *Sinophthalmus* Coq. is actually generically distinct from the species of *Phortica* and may eventually have to be placed in that group but I have not taken such a step in the present work.

We have, then, two subgenera as follows:

Subgenus **Amiota** Loew
Type: *A. humeralis* Lw.

Small to large species with uniformly dark brown, black or tannish yellow thorax, the abdomen with or without some yellowish areas. Legs usually uniformly pale, not obviously banded. Middle orbital bristle well developed, usually at least ⅔ length proclinate. Last section of 5th vein longer than posterior crossvein (5X index 1.0 or higher). Most species with milky white areas on face, on humeri and below wing bases. Examples: *minor* Malloch (U.S.), *alboguttata* Wahlberg (Europe, ? U.S.), *lacteoguttata* Portchinsky (Europe), *rufescens* Oldenberg (Europe), and *nigrescens* n. sp. (U.S.).

Subgenus **Phortica** Schiner, new comb.

Type: *A. variegata* Fall.

Mostly large species with complex pattern of grayish or darker lines and marks on mesonotum and abdomen. Legs distinctly banded. Middle orbital bristle small, less than ½ length proclinate. Never with the milky white spots as in subgenus *Amiota*. Posterior crossvein longer than last section of 5th vein (5X index usually 0.5–0.8). Examples: *oldenbergi* Duda (Europe), *africana* Malloch (Africa), *varipes* Duda (Sumatra), *annulata* Malloch (Australia), *maculiceps* de Meijere (Sumatra, Formosa), and *albavictoria* Patterson and Mainland (Mexico, U.S.).

Whereas the members of *Phortica* show considerable variation in morphology, those of *Amiota* are so similar superficially that an adequate systematic treatment is extremely difficult. The following account is far from final and an extensive study of male genitalia of many specimens, including European species, is a necessity.

The only record of larval habitat known to the writer is given by Seguy (1934) for the European *variegata*, whose larvae were found in sap of the weeping willow tree. Most species have the habit of flying into one's eyes and ears, in the manner of *Hippelates* (Chloropidae), and can be quite annoying at times. We have collected most of our specimens by sweeping a net around our heads although in extremely dry situations in the southwest species will come to banana-baited traps in fair numbers. We have never succeeded in rearing any member of the genus in the laboratory.

Key to the Nearctic species of Amiota

1. Disc of mesonotum uniformly brown, black or tan, without markings; legs not banded, usually unicolorous; 5X index 1.0 or higher; middle orbital bristle at least 2/3 length proclinate; many species with milky white areas on face, humeri and below wing bases ..... (Subgenus **Amiota**) 2
   Disc of mesonotum with pattern of grayish to blackish markings; legs strongly banded; 5X index 0.5–0.8; middle orbital smaller, less than half length proclinate; never with milky white areas as described ..... (Subgenus **Phortica**). *albavictoria* Pat. & Main.
2. With milky white areas on lower face, humeral calli and below wing bases, though these may be partly faded on pinned specimens; small to large species, black, brown or tan 3
   Definitely without such white areas; tannish to brownish flies; body length 2.0–2.5 mm., rarely larger ..... *minor* Malloch
3. Male hind femora with about 5 long yellow bristles near middle, these much longer than femoral diameter; small, black, slightly shining flies, the abdomen without yellowish areas ..... *setigera* Mall.
   Male hind femora lacking such outstanding bristles 4

4. Prosternal plates dark brown to black, the color visible even when heavily pollinose; yellowish areas on abdomen often lacking or greatly reduced; small to moderately large blackish species 5
   Prosternal plates distinctly pale yellow; usually with considerable yellow areas visible on abdominal tergites; medium to large sized flies, blackish, brownish or tan in color 8
5. Cheeks rather broad, at their narrowest point as broad as width of 3rd antennal joint; cheeks pale behind, often whitish behind the rear of the eye; large species (4.0 mm.), black, with a small amount of yellow on 1st tergite ..... *buccata* n. sp.
   Cheeks narrower, nowhere as broad as width of 3rd antennal joint; cheeks rarely noticeably paler behind, never whitish. 6
6. Arista with several rather long rays both above and below the main axis; 4th vein index 2.5 or less; rather small species (1.5–2.0 mm.), usually black with fairly dense pollinosity ..... *humeralis* Loew
   Arista with 4–5 dorsal branches above, basally, usually shorter than width of 3rd antennal joint, and with no long branches below, the main axis short pubescent to tip; 4th vein index above 2.5; generally larger black flies (2–3 mm.) with mesonotum more shining, only lightly pollinose..... 7
7. Very dark species with front, antennae, upper face, palpi and most of cheeks blackened; fore coxae and all femora dark, sometimes nearly black; mesonotum, scutellum and abdomen quite black..... *nigrescens* n. sp.
   Less noticeably black species, usually with the front lighter anteriorly, the face, palpi and cheeks brownish and the legs pale yellowish; thorax and abdomen black ..... ? *alboguttata* Wahl.
8. Large yellowish to tannish flies (3 mm. or more); front tan, only the ocellar area blackish; usually all but apical tergites with yellowish apical bands 9
   Flies of medium size (up to 3 mm.), blackish to brownish black with the front black on its posterior half or more; usually with yellowish apical bands only on two basal tergites 10
9. The two most prominent central processes of ♂ genitalia (seldom visible externally) light brown, long and slender, bifid at or near tip ..... *leucostoma* Loew
   Male genitalia with 4 prominent processes, evident externally, appearing as semi-curled rods, black in color ..... *Species B*
10. Sternopleura pale yellowish; front narrow, the width at its narrowest point slightly less than length of proclinate orbital; 3rd vein strongly diverging from 4th just beyond anterior crossvein, converging toward apex..... *Species C*
    Sternopleura largely brownish; front broader, its width greater than length of proclinate; 3rd vein not so strongly bent ..... *Species A*

Amiota (Phortica) albavictoria Patterson and Mainland.

A. albavictoria Pat. and Main., 1944. Univ. Tex. Publ. 4445:13.

The species was described from 2 males collected at La Placita near Jacala, Hidalgo, Mexico. The writer has since taken 8 specimens from Ramsey Canyon, Huachuca Mts., Ariz. This form has the habit of flying around one's head in the manner of *Amiota* and *Sinophthalmus*, and is quite similar in most respects to *S. pictus* Coq. In the latter species, however, the arista is wholly bare, lacking any branches, while in *albavictoria* there are 1–3 short dorsal branches near base, the main axis otherwise bare.

Amiota (Amiota) humeralis Loew.

A. humeralis Lw., 1862. Berl. ent. Zeit., 6:229 (Cent. II, No. 93).

The type of this species came from the District of Columbia. Dr. Sturtevant has recently examined the type and his notes have been used in placing the species in the key. However, it is still likely that two species are included here: the original from the eastern states and another form from

the mountains of the western states. I am unable to settle the point at present due to a scarcity of specimens from the east. It is not certain that the figure of the male genitalia figured by Hsu (1949, Pl. 1, fig. 5) applies to this species.

Eastern specimens in the collection of Dr. Sturtevant have been examined from Mass., N. J., and N. Y. The Texas collection has nearly 75 western specimens from the following localities: New Mexico: Cherry Creek near Silver City, Whitewater Camp near Glenwood, Magdalena Mts.; Arizona: Mt. Graham near Safford, Cave Creek in the Chiricahua Mts., Rustler Park, Ramsey Canyon in the Huachuca Mts., Madera Canyon, Tonto Creek near Payson, Mogollon Rim Road south of Flagstaff; Texas: Ft. Davis; California: Willow Creek Camp west of Arcata.

Amiota minor (Malloch).

Phortica minor Mall., 1921. Ent. News, 32:312.

The type and two paratypes were taken by Malloch at Dubois, Ill. Published records indicate that the species is widespread over the eastern United States. Our most western records are from Nebraska, Washington (Lake Wenatchee, 6 at traps), Austin, Texas, and Vera Cruz, Mexico. Steyskal (private communication) reports it from Michigan.

The clasper of the male genitalia from a specimen from New Jersey has 4–5 long, stout, blunt teeth, like fingers on a hand, while a specimen from Austin, Texas has 7 such teeth.

Amiota setigera Malloch.

A. setigera Mall., 1924. Bull. Brook. Ent. Soc., 19:51.

Malloch described the species from four specimens from Savoy, White Heath and Dubois, Illinois. One male was captured at sap exuding from an apple tree; the remainder were captured while flying around the collector's head. I know of no other published records of the species.

Amiota alboguttata Wahlberg.

A. alboguttata Wahl., 1838. K. Vet. AKad. Handl., 22.

Loew stated that he had seen this European species from North America and Malloch and McAtee (1924) report it of general occurrence in the District of Columbia region but indicate that it is doubtful if the American species is actually the same as the European one. There are a number of records of the species going by this name, however, and I have compared specimens in Dr. Sturtevant's collection from Europe determined as *alboguttata* by Frey with specimens from New York and New Jersey and could see no superficial difference. An examination of the male genitalia is essential to settle the question and European specimens have not been available for this purpose.

Amiota leucostoma Loew.

A. leucostoma Lw., 1862. Berl. ent. Zeit., 6:230 (Cent. II, No. 94).

Loew's types came from Pennsylvania. It is the common large yellow to brownish species of the eastern states. We have taken it in Va., Me., and

Mich. Specimens in Dr. Sturtevant's collection are from N. Y., N. J., and Mass. Steyskal (private communication) also reports it from Mich., and Malloch (1921) mentions it from Ill. Malloch and McAtee (1924) state that it is common around Washington, D. C., and believe that it is the same species as that described from Europe by Oldenberg as *rufescens*.

Amiota nigrescens, sp. nov.

External characters of imagines.

♂, ♀. Arista with 3–4 branches dorsally near base, usually shorter than width of 3rd antennal segment, the main axis thickly clothed with short hairs to tip and lacking any outstanding ventral branches. Front dull black throughout, shining only on narrow orbits and ocellar triangle; 2nd antennal joint brown, 3rd much darker. Face dark above, pale below (the whitish area of lower face, humeri and wing bases rarely persisting on pinned specimens), clypeus black, palpi mostly dark brown. Vibrissae weak, single, followed by a single row of weak hairs. Cheeks quite narrow, their width about equal to that of a palpus, dirty yellow to brown in color. Middle orbital 5/6 length proclinate, nearly as long as posterior reclinate and twice as far from the latter as from the proclinate. Postverticals not evident, inner and outer verticals, and ocellars well developed.

Mesonotum and scutellum shining black, very faintly pollinose. Pleurae black except for white or pale areas dorsally on humeri and just below wing bases, the dark area pollinose except for a shiny area along anterior margin of mesopleura. Prosternum dark, pollinose. Acrostichals in about 10 irregular rows; prescutellars strong. Anterior dorsocentrals short, scarcely longer than prescutellars. Anterior scutellars divergent. One humeral, 2 strong notopleurals, presutural short and thin. Two strong sternopleurals, about equal in length. Halteres white, the basal joint dark.

Legs of darkest specimens with fore coxae and femora greatly darkened, nearly black, otherwise pale yellowish except for apical tarsal joints of all legs. On paler specimens the femora are discolored though not so blackish.

Abdomen shining black, rarely a bit yellowish on basal tergite. External ♂ genitalia as illustrated by Hsu (1949, Pl. I, fig. 9). Wings clear; costal index about 2.0; 4th vein index about 2.1; 5X index about 1.2.

Body length 2.0–2.6 mm., wings about 2.5 mm. (in pinned specimen).

Distribution and types.—As far as known this species is limited to mountains in the desert regions of the west. Although we have captured nearly 50 specimens of this species, we have at present in our collection 22 pinned specimens, one mounted whole on a slide, and slide mounts of the external male genitalia of two individuals.

Holotype, ♂, No. 2164.7, from Slide Rock Campground, Oak Creek Canyon, Coconino National Forest, south of Flagstaff, Arizona, collected June, 1951 by the writer. *Paratypes* as follows: Arizona: 4, same data as holotype, Tonto Creek near Payson (11, one on slide), Long Valley, near Pine (2); New Mexico: Cherry Creek Camp, near Silver City (5). Slides

of ♂ genitalia from specimens from Oak Creek Canyon, and from Tonto Creek, Ariz.

Relationship.—Belongs to the subgenus *Amiota,* resembling *humeralis* in most superficial characters. However, the dark legs and male genitalia indicate that it is not closely related to any other species known to us.

Amiota buccata, sp. nov.

External characters of imagines.

♂, ♀. Arista long, with about 3–4 long dorsal, basal branches, their length about equal to width of 3rd antennal segment, the remainder of the main axis with rather numerous shorter hairs both above and below and laterally; arista blackish except for the main axis basal to the distal long branch, this area being pale and covered with rather thick pale hair. Antennae tan, 3rd joint darker, flattened, its length about twice its width. Front dark brownish black, dull except for the shining black ocellar triangle and orbits. Carina a rather low ridge, not nose-like. Face brownish above, broadly milky-white below to vibrissae. Clypeus and palpi black; proboscis brownish, paler basally. Posterior cheeks milky white or pale, this area extending well around to rear of head, usually leaving the middle of cheek below center of eye brown, the brown area about as long as length of 3rd antennal joint. Proclinate and posterior reclinate orbitals about equal in length, the anterior reclinate about ½ their length. Postverticals very small. Second (sometimes third) oral bristle most prominent, about twice length 1st and following ones. Cheeks rather broad, about ⅙ eye diameter.

Mesonotum, scutellum and pleurae shining black, overlaid with thin grayish pollinosity; upper surface of humeral callus and sub-alar area milky white. Acrostichal hairs in about 10 irregular rows; prescutellars strong, nearly as long as anterior dorsocentrals, the latter about half length posterior pair. Interval between dorsocentrals of each side about equal to distance between bases of two prescutellars. Anterior scutellars divergent, posterior pair cruciate. One strong humeral; 2 strong sternopleurals, the anterior only slightly shorter than posterior. Two small propleurals above base of fore coxae. Halteres white. Presutural bristle small and inconspicuous.

Prosternum dark brown. Legs uniformly pale yellowish; preapicals weak; no prominent bristles on hind femora. Abdomen uniformly shining black, or with yellowish areas on 1–2 basal tergites. Wings hyaline, without markings. Distal costal break without strong bristles. Third costal section with short, black bristles on its basal ⅗. Discal and 2nd basal cells clearly separated. 3rd and 4th veins weakly convergent apically. Costal index about 2.3–2.4; 4th vein index about 2.0; 5X index 1.0–1.1.

Length body, ♂, 3.4 mm. or more, wings about 3.6 mm. (in pinned specimen). Body length, ♀, about 4.5 mm., wings 5.0 mm.

The external ♂ genitalia are quite similar to that of the specimen from Kingston Canyon, Nevada figured by Hsu (1949, Pl. I, fig. 7) except that the present specimens show only 7 large teeth on the clasper and the finger-like process on the lower portion of the clasper is smaller.

Distribution and types.—This species is known only from mountains in central and southern Arizona and New Mexico. **Holotype**, ♂, No. 2170.5, from Mill Canyon, Magdalena Mts., near Magdalena, New Mexico, taken by the writer in June, 1951. *Paratypes* as follows: New Mexico: 6, with the same data as the holotype, Cherry Creek Camp near Silver City (4); Arizona: Long Valley in Coconino National Forest (2), Mogollon Rim Road south of Flagstaff (4).

Amiota species A.

This is apparently an undescribed species but we have had too few specimens available to settle the point. In Dr. Sturtevant's collection there are specimens from N. Y., N. J., and Mass., and the writer has taken two individuals from the San Francisco River, south of Reserve, N. Mex., which cannot be separated from the eastern specimens. The general appearance is much like that of *humeralis* but is a larger fly (usually about 3 mm.), has the prosternal plates distinctly yellow, thus contrasting with the rather shiny black mesonotum, and finally has considerable yellow areas on the basal 2–3 tergites. The specimens from New Mexico were taken at traps.

Amiota species B.

This undescribed species is quite similar to *leucostoma* in being a rather large yellow fly. The external and internal ♂ genitalia are quite different, however. We have captured less than a dozen specimens of this form in the northern tip of Maine.

Amiota species C.

This is apparently an undescribed species, known to us from a single specimen collected from Lake Hall, near Tallahassee, Florida, in June, 1950 by Dr. T. C. Hsu. A brief description follows.

Mesonotum and scutellum jet black, shining, very thinly pollinose. Abdomen black with yellow on 2–3 basal tergites. Front quite narrow, the orbits converging toward antennae, the width at narrowest point slightly less than length of proclinate orbital bristle. Arista with long thin branches above and below. Palpi pale; cheeks pale, very narrow. Fore coxae, all legs and sternopleura pale yellow. 3rd vein noticeably diverging from 4th just beyond anterior crossvein, then converging again, the costal index about 1.5. Body length about 2.5 mm. in pinned specimen.

Amiota barretti (Johnson), new comb.

Stegana barretti Johnson, 1921. Psyche, 28:59.

Johnson described this species from a single female captured at Amecameca, state of Mexico, Mexico, a town near the base of Popocatepetl. From the description one can conclude that this fly was largely black, with the typical milky white areas, about 3.5 mm. in length. It cannot be placed in the key to species.

CACOXENUS Loew

1858. Wien. ent. Monatschr., 2:217.

Genotype: *C. indagator* Loew.

= *Paragitona* Kröber, 1912. Zeit. wissen. Insek., 8:235. (Type: *P. obscura* Kröber = *C. indagator* Lw.)
? = *Gitonides* Knab, 1914. Insec. Inscit. Menstr., 2:165.

In August, 1951, the writer captured 24 specimens which appear to belong to this genus. The localities and number of specimens are as follows: Dungeness River, Olympic Nat. Forest, Wash. (4); Lake Wenatchee, Wash. (17); Tyee Springs near Carson, Wash. (2); Polally Forest Camp, Mt. Hood Nat. Forest, Ore. (1). All of these were taken by sweeping over muddy ground along streams or lakes, and all were males. I have been unable to decide whether these represent an undescribed species or not, and no specific name is being applied to them at present.

In working with the above specimens, it became apparent that the genus as understood by Duda, Hendel and Seguy is not at all the same as the group discussed by Melander (1913). The situation is also complicated by the fact that some authorities place the genus in the Milichiidae (as does the Zoological Record at present), while others insist it belongs to the Drosophilidae. Hendel (1933) states that he first placed it in the latter family in 1917 and it is certain that the specimens available to the writer belong here, and are obviously related to the *Amiota-Phortica* assemblage. Also belonging to this group are: *indagator* Loew (Europe), *exiguus* Duda (Europe), *punctatus* Duda (Formosa; according to Hendel, *op. cit.*, = *Gitonides perspicax* Knab from the Pacific area), *inquilinus* Hendel (Europe), and *argyreator* Frey (Finland). The other species going under this generic name is *C. semiluteus* Loew from Cuba. Dr. Sturtevant, who has examined the type at Harvard, states (private communication) that this fly has the disc of the scutellum hairy and mesopleurae bristled, thus removing it from the family.

Several species of the genus are believed to be parasites. *C. indagator* has been reared from nests of several Hymenoptera, e.g., *Osmia* and *Chalicodoma*. Kröber (1912), however, believes that the larvae feed on the pollen which is carried into the cells by the bee. *Gitonides perspicax* Knab has been reared several times from larvae feeding upon mealybugs of the genus *Pseudococcus*.

CHYMOMYZA Czerny

1903. Zeithschr. Hym. Dipt. 3:199.

Genotype: *C. fuscimana* (Zetterstedt).

Most members of this genus are not readily attracted to banana-baited traps but are found around peeled areas on tree trunks, especially aspen, alder, fir and pine, in our experience. They do not seem to be attracted to slime flux exudations, however. We have had considerable success in raising these species in the laboratory where it has been observed that non-

yeasted food is preferable. All the species observed have the habit of constantly waving the wings. Courtship is usually very simple, the males often flying rapidly to the female and attempting copulation without any true courtship other than wing-waving. The males use the spiny fore femora to hold on to the wings of the female, and they have also been observed fighting among themselves, using the front legs like boxers.

Key to the Nearctic species of Chymomyza

1. All legs yellow; wings somewhat whitish at tip 2
   Fore femora, tibiae and one or more tarsal joints dark, other legs paler; wings whitish at tip or not 4
2. Wings with a pattern of 3 dark areas, one at apex of 1st vein, one across middle and posterior crossvein, and one below apex of 2nd vein; wing tip white ..... *amoena* (Loew)
   Wings without the described pattern though the costal cell is dark and there may be a black spot near wing tip 3
3. Wings with a distinct black spot just below and beyond apex of 2nd vein; oral margin black, strongly contrasting with the pale yellow face and antennae ..... ? *distincta* (Egger)
   Wings without the described apical black spot, with only the costal cell darkened; oral margin discolored but not black and contrasting with the face ..... *Species A*
4. Wings clear, the costal cell not darkened; anal plates and ventral lamellae of ♂ noticeably enlarged, the former quite long-haired, the latter chitinized and strongly protruding posteriorly; converging proclinate orbitals situated only slightly nearer the anterior reclinates than the posterior reclinates or equidistant between them; fore metatarsus black, the apical 4 joints pale ..... *caudatula* Olden.
   Costal cell darkened; male genitalia often prominent but not so remarkably elongated; proclinate orbitals usually much nearer anterior reclinates than posterior ones 5
5. Basal one or two segments of fore tarsi black, the remaining apical joints contrastingly whitish or yellowish 7
   Fore tarsal segments all dark or becoming gradually paler apically without a strong contrast between dark and light joints 6
6. Fore coxae of ♂ heavily long-haired along inner surface; fore legs of ♂ usually dirty brown, becoming paler apically, those of ♀ generally much darker with only the apical joint pale; larger species, usually 3–3.5 mm. ..... *coxata* n. sp.
   Fore coxae of ♂ not long-haired; all segments of fore tarsi dark on both sexes; smaller species, 2–2.5 mm. ..... *aldrichi* Stvt.
7. Only fore metatarsus black to brown, the 4 apicals pale 8
   Two basal tarsal joints dark, the 3 apicals pale 9
8. Wings whitish at tip; ♂ fore femora with stout row of spinous bristles along inner edge, becoming longer basally, the longest ones distinctly longer than the tibial diameter ..... *procnemis* (Will.)
   Wing tip not whitish; ♂ fore femora with a row of short, rather thin spines along inner edge, none of them as long as tibial diameter ..... *procnemoides* n. sp.
9. Front shining black with iridescent pollinosity, paler near antennae; mesonotum and scutellum shining black with similar pollinosity; face pale yellow ..... *mexicana* Whlr.
   Front tan, the orbits and ocellar triangle darker brown; mesonotum and scutellum dark blackish brown, subshining, with light pollinosity; face yellow above, brown along oral margin ..... *Species B*

Chymomyza amoena (Loew)

Drosophila amoena Lw., 1862. Berl. ent. Zeit., 6:230 (Cent. II, No. 96).

The type material came from the District of Columbia. Sturtevant (1921) lists it from most of the eastern states. The distributional limits, according to our collections, are: Minn., Nebr., Utah, Ariz., Texas, and

several places in Mexico. Judd (1949) reports two specimens from Ontario, Canada.

C. amoena is attracted to traps quite commonly and can easily be reared on laboratory food. I know of no record reporting this species from peeled tree trunks but Sturtevant *(op. cit.)* states that it has been bred from walnut and butternut husks and from acorns.

Chymomyza procnemis (Williston).

Drosophila procnemis Will., 1896. Tr. Ent. Soc., London, Pt. 3:412.

At least two species are being confused under this name. Williston's species has the tip of the wing whitish but the more common species of the United States lacks this character and differs in several other respects. It is therefore being described below as new.

The types of *procnemis* were from the West Indies and it seems to have mainly a subtropical distribution. The writer has examined material from Cuba, Mexico, Fla., Ala., south and west Texas, and specimens from Oahu, Hawaii sent to us by Mrs. Sarah B. Pipkin and by D. E. Hardy. One specimen labelled Algonquin, Ill., 6.1.94 (U. S. National Museum collection) seems also to be this species. It is fairly certain, however, that most northern records for *procnemis* are applicable to the following species. True procnemis comes to traps readily and can be easily reared in the laboratory.

Chymomyza procnemoides, sp. nov.

External characters of imagines.

♂, ♀. Arista with about 3 dorsal and 2 ventral branches in addition to the terminal fork. Front largely pale yellowish tan, orbits and ocellar triangle more shining but not appreciably darker. Antennae yellow, 3rd joint no darker, 2nd joint with 1–2 strong bristles. Face pale tan, becoming grayish brown along oral margin and distinctly blackish around bases of first one or two oral bristles on males, on some females scarcely darker here. Clypeus, palpi and proboscis pale yellow. Cheeks pale yellow except on vibrissal area, very narrow at this point, broader behind, its width at narrowest point about 1/12 greatest eye diameter. All orals stout, the 2nd about ⅔ length 1st. Proclinate orbital about ¾ length anterior reclinate, the latter about as long as posterior reclinate; proclinates somewhat convergent. Postverticals minute.

Mesonotum, scutellum and pleurae uniformly pale tan, the notopleural region sometimes a bit darker. Acrostichals in 8 irregular rows just before anterior dorsocentrals; no prescutellars. One strong humeral; a small propleural present just above fore coxae. Anterior sternopleural nearly ⅔ length posterior one. Halteres white. Fore femora, tibiae and metatarsi black, legs otherwise pale yellowish. Inner edge of ♂ fore femora with a row of 6–10 short, stout spines. Abdomen shining black, the genital area paler.

Wing with costal cell and costal vein dark, otherwise clear hyaline and lacking a whitish area at tip. Distal costal break with one stout bristle, the ventral one shorter and thinner; 3rd costal section with heavy spines along

its basal ⅘. Costal index about 1.5–1.6; 4th vein index about 2.6; 5X index about 3.0.

Length body, ♂, 2.0 mm., wings, 2.0 mm. (in pinned specimen). Body length, ♀, 2.5 mm., wings, 2.2 mm.

Distribution.—This species is known certainly from Texas, N. Mex., Ariz., Minn., Mich., Ind., Ill., Va., and N. Y. In addition, it is likely that many northern records for *procnemis* are applicable to this species.

Types.—**Holotype**, ♂, No. 2152.6, from Whitewater Campground, Glenwood, N. Mex., 6.8.51, collected by the writer. *Paratypes:* New Mexico: 5, with same data as holotype, Silver City (2); Arizona: Ramsey Canyon, Huachuca Mts. (1), Cave Creek, Chiricahua Mts. (1), Patagonia (8), Madera Canyon, Coronado National Forest (1); Texas: Austin (1), Dallas (2: A. H. Sturtevant); New York, N. Y. (1: A. H. Sturtevant); Michigan: Battle Creek (1: J. M. Aldrich, USNM); Indiana: La Fayette (1: J. M. Aldrich, USNM); Illinois: Urbana (1: J. M. Aldrich, USNM); Virginia: Dead Run, Fairfax Co. (1: R. C. Shannon, USNM).

Notes.—In comparison with *procnemis*, the present species is usually a little smaller, lacks the whitish area at wing tip, has the lower part of the face of males distinctly darkened, sometimes present also on females, and the row of stout bristles along inner edge of fore femora of males shorter than the tibial diameter. The external male genitalia (anal plate, genital arch, clasper) of the two are practically the same (see fig. 7, Pl. II, Hsu, 1949), but the internal sclerotized processes of the two differ in several details.

This species comes to traps rather poorly, our largest collection being 13 specimens from a single locality. It is extremely difficult to raise in the laboratory; only once have we succeeded in getting an F_1 generation and these flies failed to breed further.

Chymomyza aldrichi Sturtevant.

C. aldrichii Stvt., 1916. Ann. Ent. Soc. Am., 9:325.
= *C. tetonensis* Wheeler, 1949. Univ. Tex. Publ. 4920:163. New Syn.

The writer believed *tetonensis* to be distinct from *aldrichi* since the description of the latter implied that only the basal tarsal segment of the fore legs was dark. However, I have compared a paratype in Dr. Sturtevant's collection and Mr. Willis Wirth has kindly examined the holotype in the collection in the National Museum, and on both the entire fore tarsi are dark. Thus *tetonensis* must be considered a synonym.

C. aldrichi was described from Idaho. Collectors from this laboratory have taken it in the following states: Calif. (northern), Ore., Wash., Ida., Wyo., Utah, Colo., Ariz., and N. Mex. Although a number of these specimens were attracted to traps, most were captured from peeled areas on trees, mainly aspen, fir and pine. In addition, we have a stock of this species from Itasca Park, Minn., collected by Dr. H. T. Spieth, who found the larvae in bark of *Populus grandidentata* and *P. tremuloides*, and we have examined a specimen from Caratunk, Maine (A. H. Sturtevant, Aug.,

1950). This species can be raised on laboratory food but requires constant care.

It should be pointed out that the figure of the external male genitalia of *tetonensis* (Hsu, 1949, Pl. II, fig. 5) is referable to *aldrichi*, while the figure labelled as *aldrichi* was apparently a misidentification and we do not know to which species it belongs.

Chymomyza caudatula Oldenberg.

C. caudatula Old., 1914. Arch. f. Naturg., 80 A 2:14.

This European species has been previously reported from Washington. I have not seen European specimens but Melander and Sturtevant seem agreed that our form is the same. We have collected it in the following states: Calif. (northern), Ore., Wash., Wyo., Utah., and Ariz. It seems to have a boreal distribution also, for Steyskal (private communication) reports it from Michigan and I have examined a specimen from Maine in the USNM collection. The species can be raised in the laboratory, but like many other species, it has such a long larval and pupal life that mortality is rather high. It is attracted to banana traps in small numbers, most of our specimens being captured either on damaged trees or at windows.

Internal characters.—Spermathecae with small, dense black centers; parovaria only slightly smaller. Ventral receptacle a short blunt tube bent back on itself at about the middle, the basal half with a large lumen, the distal half with a very narrow lumen. Testes of aged male yellowish orange, roughly V-shaped with a narrow basal arm and a broader distal arm, much like that in *Drosophila victoria* (see Patterson, 1943, fig. 11). The paragonia are also similar to those of the latter species and in one specimen examined these structures contained large numbers of sperm. Ejaculatory sac with no apparent diverticula, the vas deferens from testis to ejaculatory sac unusually long and thick, especially basally, thicker than in any other species we have examined. On the basis of the internal characters and certain external features, this species seems to be unrelated to any other species known to us.

Chymomyza mexicana Wheeler.

C. mexicana Whlr., 1949. Univ. Tex. Publ. 4920:162.

This species is known only from the type from Puebla, Mexico. A specimen from Pacific Grove, Calif. (*Species B*, below) is quite similar.

Chymomyza coxata, sp. nov.

External characters of imagines.

♂, ♀. Arista with about 3 dorsal, 2 ventral branches in addition to the terminal fork; 2nd antennal joint with 2 strong bristles and numerous smaller ones. Front tan, orbits grayish pollinose, ocellar triangle blackish. Antennae tan, darker on outer sides; face tan, becoming more or less dirty gray below along oral margin; face not carinate. Cheeks yellowish white, their width 1/10 to 1/12 greatest diameter of eyes. Row of oral bristles

strong, the 2nd oral a little over half length 1st. Palpi pale yellow, without outstanding bristles; proboscis pale. Orbitals arranged as usual in the genus, the proclinates converging somewhat, the anterior reclinates placed well in front of them; proclinate about 5/6 length anterior reclinate, this about 5/6 length posterior reclinate, the latter about midway between anterior reclinate and inner vertical; a small hair usually present between proclinate and posterior reclinate. Postverticals small.

Acrostichals in 8 rows just anterior to 1st dorsocentrals; no prescutellars. Anterior scutellars weakly divergent or straight. One strong humeral. A small hair just above base of fore coxa. Two strong sternopleurals, the anterior one about 3/4 length posterior. Mesonotum usually tannish brown with indistinct median blackish area, the entire disc covered with thin grayish pollinosity. In some individuals the blackish area is more extensive and may rarely cover most of the disc. Notopleural area usually darker than dorsum, meso- and sternopleurae pale tan. Basal joints of halteres discolored, the knob whitish. Abdominal tergites uniformly black, faintly shining under a light pollinosity.

Prosternum, fore coxae and trochanters and all of 2nd and 3rd legs uniformly pale yellow; fore femora, tibiae and metatarsi black, the next tarsal segment usually about as dark as the 1st, the remaining joints becoming increasingly paler towards tip, the apical one thus more or less yellowish. Fore coxae and femora of female as usual; fore coxae of male quite densely haired and bristled along their length on inner side; fore femora also heavily armed with hairs and bristles along inner surface, a row of about 10 especially stout spines fitting against tibia when legs are bent, the spines of this row about as long as tibial diameter.

Wings clear, the costal cell darkened. Third costal section with heavy bristles along its basal 3/5. Distal costal break with one strong bristle, the ventral one distinctly shorter and thinner. Costal index 2.0–2.2; 4th vein index 2.3–2.5; 5X index 3.3–3.5.

Length body, male: 2.8 mm.; wings: 2.6 mm. (in pinned specimen).

Female, body: 3.2 mm.; wings: 2.8 mm.

Internal characters of imagines.

Spermathecae with dark sclerotized centers and thick stalks, their point of origin noticeably posterior to bases of spermathecae. Ventral receptacle rather short, with about two loops wound as in *Hirtodrosophila,* the basal portion about twice as thick as remaining 9/10. Attached by muscle and tracheae to the dorsal surface of the vagina is a large, roughly bi-lobed sac, faintly greenish-brown in color, apparently comparable to the similar structure reported by Wheeler (1947) in members of the *willistoni* group of *Drosophila.*

Testes irregularly coiled, with about 2 large, pale yellow inner coils and 2–3 thin, pale outer coils; paragonia large. Sperm pump brownish in color, without diverticula.

Other characteristics, relationship and distribution.

Eggs and puparia.—Eggs not greatly different from the usual *Chymomyza* type, greatly flattened at the micropylar end, with 6 strong but short filaments on each side, all bent strongly toward micropyle. Pattern of follicle cells distinct, resulting in numerous irregular longitudinal furrows on the surface. Puparia as usual, 3.5–4 mm. in length, the posterior spiracles strongly divergent and black at tip.

Distribution and types.—Known from 19 specimens collected in the higher mountains of Colorado and Wyoming, and their descendants raised in the laboratory. **Holotype**, ♂, No. 2057.2, from about 30 miles north of Durango, Colo., collected by the writer Aug., 1950. *Paratypes* as follows: Colorado: 9, with the same data as the holotype; Wyoming: Teton Pass, Targhee National Forest (2), Gros Ventre Road, east of Moose (8). One paratype ♂, from Colorado, is being placed in the National Museum collection.

Notes.—None of the wild specimens came to traps. The Colorado collection was made from aspen trees rather recently felled and partially peeled by beavers. The specimens from Gros Ventre Road, Wyo., were found on an aspen blown over by wind, while those from Teton Pass were taken from aspen which had been peeled by the writer in order to attract members of the genus. At all localities, *C. aldrichi* was taken at the same aspen trees.

It is possible to raise this species in the laboratory but it is difficult since the long larval life usually results in the culture medium becoming hard and moldy before the larvae are ready for pupation. A single generation in the laboratory takes a month or longer. This species is not obviously related to any other North American species.

Chymomyza ? distincta (Egger).

Drosophila distincta Egger, 1862. Verh. zool. bot. Ges., 12.

Ten male specimens of what may be this European species were taken by the writer and Wm. Heed from partially peeled fir logs and chips at the Dungeness Fork Forest Camp, Olympic National Forest about 10 miles south of Sequim, Wash., Aug., 1951. Although these specimens trace to *distincta* in the available keys, a comparison with the original description or with determined European specimens has not been possible so the name must remain tentative.

A brief description of these flies is as follows: mesonotum, scutellum, front and upper face pale tannish, lower face with a broad black band above oral margin; cheeks pale; abdomen black except basally. All legs pale yellow, fore femora of ♂ with a dense series of stout bristles along inner edge, many of which are longer than the tibial diameter. Wings with costal cell blackish, tip whitish, and with a distinct black cloud in submarginal cell just below and beyond apex of 2nd vein. External male genitalia not especially large but noticeably long haired.

European writers imply that the apical wing cloud is sometimes absent in *distincta;* this might easily be true on teneral individuals or it might be a female characteristic. On all of the above males the cloud is quite distinct.

Chymomyza species A.

This unnamed species is known from two specimens collected at Timagami, Ontario, Canada (1. IX. 1932, A. W. A. Brown. USNM collection). It is a pale tan species with the abdomen somewhat darker. The costal cell is darkened and the wing tip whitish, the wings without other markings. All legs are uniformly pale yellowish tan.

Chymomyza species B.

This species is known at present from a single specimen from Pacific Grove, California (A. H. Sturtevant). It may be characterized briefly as follows: mesonotum and scutellum sub-shining blackish brown, front tan, the orbits and ocellar triangle shining, darker. Face yellowish centrally, brown along oral margin. Fore femora, tibiae and 2 basal tarsal joints blackish. Fore femora of male with a row of short, stout spines on inner edge, 15–18 in number, none as long as tibial diameter. Costal cell brown.

CINDERELLA Steyskal

1949. Bull. Brooklyn Ent. Soc. 44:134–137.

Genotype: *C. lampra* Steyskal.

This, the only described species in the genus, is not known to belong to this family. It is represented by a single ♀ from Ada, Okla. (July) and, according to Steyskal (1949), keys to the Drosophilidae in all the more common family keys. As he points out, the shining black body surface is not Drosophiline and the outstanding character of this group, the presence of a proclinate orbital bristle, is lacking. It possesses, however, convergent postverticals, strong preapical tibial bristles, bare mesopleura, and the eight acrostichal rows and thoracic bristles are as usual in this family. His figure of the wings shows the costal index to be about 2.7.

CLADOCHAETA Coquillett

1900. Proc. U. S. Nat. Mus., 22:263.

Genotype: *C. nebulosa* Coq.

This is the only member of the genus found in our area and is primarily a tropical species which reaches into Florida. Sturtevant (1921) lists it from Florida, Mexico (Vera Cruz), Porto Rico and Cuba. I have examined his specimens from the latter locality. Curran (1928) reports it from St. Croix Island, of the Virgin Islands. The types were collected in Porto Rico.

CLASTOPTEROMYIA Malloch

1924. Proc. Biol. Soc. Wash., 37:31.

Genotype: *C. inversa* (Walker).

This genus has recently been reviewed in detail by Frota-Pessoa (1947) who places *Diathoneura* as a synonym. I have discussed briefly the difficulties of this treatment under that genus.

Key to the Nearctic species of Clastopteromyia

1. Mesonotum with an indistinct darker central stripe and traces of similar faint stripes between the former and the lateral margins; 4th vein index about 1.5; 5X index but little more than 1.0 ..... *floridana* Malloch

   Mesonotum uniformly tannish, without any indication of darker longitudinal stripes; 4th vein index about 2.0; 5X index 1.5 or higher ..... *inversa* (Walker)

Clastopteromyia floridana Malloch.

C. floridana Mall., 1924. Proc. U.S. Nat. Mus., 66:10.

This species is known only from the types from Florida.

Clastopteromyia inversa (Walker).

Drosophila inversa Walk., 1861. Tr. Ent. Soc. London, 5:331.

This species has a rather extensive distribution: in the New England area of the east, south to New Jersey, Maryland and Virginia, across the northern states where it is known from Indiana, Illinois and Minnesota, and reappears in the west where it is rather common in southern California. I have seen specimens in the collection of Dr. A. L. Melander from Bellingham, Wash., and Steyskal (private communication) reports that he has specimens from Mich. and British Columbia.

The species is remarkable in that the larvae live as ectoparasites on nymphal spittle insects of the genus *Clastoptera* (Cercopidae). Some doubt has been expressed by Malloch and others concerning this habit, inferring that the fly larvae probably utilize the froth material for food rather than the nymphs themselves. The observations of the writer and Dr. Sturtevant have convinced us that the larvae feed directly on the *Clastoptera* nymphs; in examining spittle masses for larvae they were invariably found lying on the abdominal dorsum of the nymph with the mouthparts inserted between two adjacent tergites, usually the 3rd from the rear. The posterior spiracles are surrounded by a large bubble. We were never able to rear larvae to adults when the nymphs were removed from the spittle mass, and eggs and larvae placed on standard Drosophila food failed to develop.

The eggs, about 0.85 mm. long, are evenly rounded, elongate, and show no visible follicle cell markings. There are no terminal filaments but at one end is a tiny knob, presumably the micropyle. According to Sturtevant (unpublished notes) and my own observations, the older larvae have the black ventral hooklets aggregated into small areas that lie on the surface of rounded button-like projections, one such area on each side of the six anterior abdominal segments. In crawling, these buttons are used as feet, each pair in turn being projected on the end of a "leg," giving a general impression much like that of the use of pseudopods by caterpillars. There are about 30 hooklets to each button.

The host cercopid for *inversa* in the eastern United States seems always to be *Clastoptera obtusa* which is usually found on the alder (*Alnus* sp.). In California, *Clastoptera lineaticollis* serves as host and is found on a variety of plants, including species of *Baccharis, Artemisia, Senecio, Alnus,* etc. A summary of the known host plants is given by Doering (1942). A

similar habitus has been described for *C. paradoxa* Lamb, the nymphal cercopids living on casuarine trees in Trinidad.

DETTOPSOMYIA Lamb

1914. Tr. Linn. Soc. London, 2 ser., 16:349.

Genotype: *D. formosa* Lamb.

This genus shares with *Mycodrosophila* the exceptionally deep cleft at the apex of the subcostal vein, but differs noticeably in the complex pattern of the mesonotum and smaller size. The only species in our region is probably an introduction.

Dettopsomyia nigrovittata (Malloch).

Drosophila nigrovittata Mall., 1924. Proc. Linn. Soc. N.S.W., 49:352.

Described from Australia by Malloch, this small dark species with an intricate mesonotal pattern was reported by Wheeler (1951) from California. A colony was apparently established in a rotting area on a large stem of a banana tree at Monrovia, Calif., and it seems likely that it is a rather recent introduction. The species can be raised on standard Drosophila food but requires constant attention.

Internal characters.—Spermathecae large with large sclerotized centers, narrower at base, broadly swollen just before apex, their central channels large; parovaria much smaller, with hollow centers. Ventral receptacle quite long and tightly coiled. Testes light brownish-orange with 7 colored loose outer coils and a long slender inner tube which is only slightly coiled; parogonia large. Ejaculatory sac with two short, blunt, thick diverticula. Anterior Malpighian tubes free, their common stalk about ⅓ their total length, the posterior pair apposed at the tips but without a continuous lumen, their common stalk a little less than half their total length. Both pairs are shorter than in typical *Drosophila.*

The eggs are quite small with 4 short filaments. The puparia are pale yellowish-tan, each anterior spiracle with about 9 branches, most of them as long as the spiracular stalk, the latter plus its branches nearly ¼ length of puparium. Posterior spiracles weakly divergent apically. Length 2 mm.

DIATHONEURA Duda

1924. Arch. Naturg. 90 A 3:180.

Genotype: *D. taeniatipennis* Duda.

Frota-Pessoa (1947) placed this group as synonymous with *Clastopteromyia* Malloch after comparing a series of species and additional published descriptions. As indicated in his summary of this joint group, there is considerable variation among the various species and it may yet be discovered that two or more genera are being included in it. In this connection it seems of interest that Malloch (1934a), in discussing *Diathoneura*, states that he is "convinced from an examination of Duda's papers

that he placed in *Diathoneura* a number of species that belong . . ." to the genus *Calopterella* Coq. (= *Diastata auct.;* see remarks in introduction). Of the species which I have seen, referable to this portion of the family, typical *Clastopteromyia* possess a rather well-developed pair of postvertical bristles, while the supposed species of *Diathoneura* have these bristles greatly reduced in size, nearly absent; whether this separation will always hold I do not know. For the present, it seems best to include the genus in the key by the use of this character to enable its users to identify material which will not run to the genus *Clastopteromyia.*

We have seen a specimen of an undescribed species from Coatepec, Vera Cruz, Mexico (D. L. Lindsley; Sturtevant collection) which keys here. It is likely that other species will be found since many are described from Costa Rica and other neotropical regions.

GITONA Meigen

1830. Syst. Beschr., 6:129, 215.

Genotype: *G. distigma* Meigen.

The type species has been reared from flowers of *Sonchus arvensis* and is believed to be aphidophagous. According to Seguy (1934), two African species have equally interesting food habits, *G. lesnei* developing within injured capsules of cotton plants, and *G. paolii* apparently living on the mealybug *Pseudococcus sacchari.* None of the three American species has been found to be carnivorous, although one of them, *bivisualis,* has not been reared and hence might have such habits. Both *G. americana* and *sonoita* live in species of cacti. Both *americana* and *bivisualis* have been raised on laboratory food for one generation but they seem loathe to mate and deposit eggs under these conditions.

Key to the Nearctic species of Gitona

1. Arista rather long pubescent or short plumose, the hairs several times longer than the diameter of the main axis; legs with darker bands apically on femora and basally on tibiae; pleurae with rather distinct longitudinal stripe; ♂ genitalia with a tooth-bearing clasper; eyes, in life, irridescent yellowish green below, purplish yellow above ..... *bivisualis* Pat.

   Arista quite short pubescent, the hairs scarcely longer than diameter of main axis; legs pale, without bands; pleurae lacking longitudinal stripe; ♂ genitalia without clasper, but with a single tooth on lower margin of genital arch; eyes, in life, yellowish green throughout 2

2. Second complete tergite with dark apical band interrupted in middle and laterally, leaving the dark marginal band unconnected with the median one; 4th vein index about 3.9; width of 3rd antennal segment about 2/3 its length ..... *americana* Pat.

   Second complete tergite with dark apical band interrupted only in mid-line, the marginal area broadly connected with the median portion; 4th vein index about 3.0; 3rd antennal segment broad and flat, its width about ½ its length ..... *sonoita* Whlr.

Gitona americana Patterson.

G. americana Pat., 1943. Univ. Tex. Publ. 4313:33.

This is a widely ranging species, known from Florida, Oklahoma, Texas, New Mexico, Arizona, California and Mexico. In central Texas it breeds

in the ripe fruits and rotting stem sections of *Opuntia lindheimeri,* and uses related species of the *"platyopuntia"* group in other areas. The eggs are similar in structure to those of *Sinophthalmus pictus* (Fig. 1). The species comes to banana-baited traps in moderate numbers.

Gitona bivisualis Patterson.

G. bivisualis Pat., 1943. Univ. Tex. Publ. 4313:35.

This species has been taken at traps in several localities in central Texas; all other known records are as follows: Arizona: Parker (2), Patagonia (12); California: Borrego State Park (1), Kernville (3); Sonora, Mexico (1); Soledad, Cienfuegos, Cuba (1; collected by J. I. Townsend). The larval habits of this form are not known. The eggs are similar to those of *americana.*

Gitona sonoita Wheeler.

G. sonoita Whlr., 1949. Univ. Tex. Publ., 4920:158.

The two types came from near Patagonia, Ariz., where they were attracted to traps. The other known records are: Mangus Canyon near Silver City, N. Mex. (1, at trap); Austin, Texas (8), reared from a rotting specimen of the cactus, *Echinocereus caespitosus.* This cactus is a fairly common plant of the limestone hilltops of the Edward's Plateau in central Texas. On the higher deserts of west Texas, New Mexico and Arizona the Rainbow Cactus, *Echinocereus rigidissimus,* is rather common and may be the host plant in these areas.

LEUCOPHENGA Mik

1886. Wiener Entomolog. Ztg. :317.

Genotype: *L. maculata* (Dufour).

This is apparently the second largest genus in the family with more than 100 described species over the world. The tropical areas, *e.g.,* central Africa, Central and South America and the islands of the Pacific are especially rich in species while temperate regions have relatively few species. Until now, only two species have been reported from the U.S., *varia* and *maculosa,* but recent collections by members of the Texas laboratory in the mountains of the west have turned up four other species, one of them described previously from Mexico. These four differ from the usual species in having rather well developed postvertical bristles and the costa apparently reaching the apex of the 4th vein, though weak beyond the 3rd. Of the various segregates which have been suggested for the family (cf. Sturtevant, 1921, p. 59), these aberrant species would most likely trace to *Neoleucophenga* Oldenberg, and one of our new species *(trisphenata)* is quite similar to *N. quinquemaculata* (Strobl), the type of that group.

As far as is known, all members of the genus are fungus breeders, and the majority of the species have been collected from fungi. In our own collections, placing putrid fungi in bait cans has increased the number of specimens considerably, but in times of severe drought, when it may be

assumed that the usual feeding sources are absent or nearly so, these flies will come to banana-baited traps fairly readily. None of the species seems easily reared in laboratory vials, however, and they usually become stuck in the food within a few minutes.

Key to the Nearctic species of Leucophenga

1. Postvertical bristles minute, much smaller than orbitals 2
   Postverticals well developed, nearly as large as proclinate orbital 5
2. Wings clear, without clouds; palpi black; abdomen yellow with a central and two lateral blackish longitudinal stripes..... *paludicola* Pat. and Main.
   Wings with dark clouds along some longitudinal veins or on crossveins or both 3
3. Wings clouded at apex of 2nd vein and over both crossveins..... *maculosa* (Coq.)
   Wings clouded at apex of 2nd vein but without clouds on crossveins 4
4. Abdomen largely yellow with a pattern of dark bands; palpi pale yellow ..... *varia* (Walker)
   Abdomen largely black, the basal tergites with narrow yellow areas basally; palpi black ..... *bimaculata* (Loew)
5. Mesonotum with four dark longitudinal stripes, sometimes poorly defined, fading behind; scutellum blackish on basal ⅔, the apex yellowish; marginal cell with 3 clouds: one at costal break, one near middle of marginal cell and one over apex of 2nd vein ..... *pulcherrima* Pat. and Main.
   Mesonotum tan, without markings, or with spots at bases of hairs and bristles, these sometimes fused into an irregular median stripe; scutellum pale basally; marginal cell with 1–2 clouds 6
6. Arista with dorsal branches only, none below main axis; hairs and bristles arising from punctate spots but lacking a pattern; meso- and pteropleurae largely discolored ..... *guttata* n. sp.
   Arista with several ventral branches; mesonotum uniformly pale tan or the bristles arising from spots which are largely fused in midline to form an irregular stripe; pleurae pale or with at most a narrow stripe..... 7
7. Mesonotum with median longitudinal stripe, irregular in outline; mid-frontal area with dark brown areas separated by a pale line; pleurae often with a faint darker stripe; "knees" darkened; abdominal pattern distinct..... *trisphenata* n. sp.
   Mesonotum uniformly pale tan; front uniformly tannish to brownish; pleurae pale; "knees" pale; abdominal pattern scarcely evident..... *montana* n. sp.

Leucophenga maculosa (Coquillett).

Drosophila maculosa Coq., 1895. Proc. Acad. Nat. Sci. Phila. :317.

This species is easily recognized by the large, flat, broad palpi, and the distinct black clouds over crossveins, just before tip of 2nd vein, and at apex of 1st vein extending posteriorly to 4th vein. It is widely distributed over the eastern half of the U.S., and is fairly common within the area bounded by New York and Minnesota on the north, Nebraska and Kansas to Texas on the west. It is more common in the southeastern states. Other published records are as follows: Lake Tahoe, Nevada (Patterson, 1943); Cuba, Haiti, Peru (Sturtevant, 1921); Argentina (Malloch, 1934a); Kartabo, British Guiana (Curran, 1934). The types were from Florida.

Leucophenga varia (Walker).

Drosophila varia Walk., 1849. List Dipt. Ins. :4.

This is primarily a species of the southeastern states, the types being from Georgia. We have seen specimens from as far north as Massa-

chusetts, as far west as Nebraska and Texas, and as far south as Neuvo Leon, Mexico. Our collections indicate that it is a much commoner species than is *maculosa*.

The internal structure of this species has not been described in detail. Dissections reveal the following:

Testes of aged male dark lemon yellow with about 3 outer coils and 2–3 inner, irregularly twisted coils. Ejaculatory sac simple, without diverticula. Vas deferens, leading to sperm pump, much broader and stouter than in *Drosophila*. Spermathecae elongate, longer than wide, with long, narrow, irregularly annulate sclerotized centers; their stalks are thick with a short S-shaped bend at the point of attachment with the chitinized central tube. Parovaria elongate oval, rather thin-walled and with a large central cavity, their size equal to or slightly surpassing that of spermathecae. Ventral receptacle a highly coiled series of loops closely appressed to vagina near base of oviduct.

Leucophenga paludicola Patterson and Mainland.

L. paludicola Pat. and Mainl., 1944. Univ. Tex. Publ. 4445:514.

This species was described from 20 specimens from Patzcuaro, Mich., Mexico. We know of no additional records. *L. ornativentris* Kahl from Bolivia is also said to possess unmarked wings and a yellowish abdomen with three longitudinal black bands, but has yellow palpi and is much smaller, being only slightly over 2.0 mm. in length.

Leucophenga bimaculata (Loew).

Drosophila bimaculata Lw., 1865. Berl. ent. Zeit., 9.

This species was described from Cuba, and is included here since we have taken a single specimen in Oaxaca, Mexico.

Leucophenga pulcherrima Patterson and Mainland.

L. pulcherrima Pat. and Main., 1944. Univ. Tex. Publ. 4445:14.

This species was described from four specimens from Jacala, Hidalgo, Mexico. We have since taken it at four localities in western United States as follows: New Mexico: Cherry Creek Campground near Silver City (5), Whitewater Campground near Glenwood (7); Arizona: Cave Creek in the Chiricahua Mts. (1), Mogollon Rim Road south of Flagstaff (2). These were collected in June, July and August. The above specimens agree with the description and the type except that the male is described as having the palpi and 3rd antennal joints black (the female is not described for these characters) while in the present series these structures are pale in both sexes. However, the description points out that considerable color variation existed in the Mexican series, with a light phase, a dark phase (described), and intermediates. There may well be two species concerned here.

Leucophenga trisphenata, sp. nov.

External characters of imagines.

♂, ♀. Front pale yellowish tan, ocellar triangle and mid-frontal area burnt blackish, on most specimens with a fainter yellowish line running anteriorly along midline from ocelli. Antennae pale tan, 3rd joint slightly darker apically. Face yellowish, flat, not at all carinate. Cheeks, clypeus, proboscis and palpi pale yellow, the latter with 5–6 strong ventral bristles, none apical. One strong vibrissa. Cheeks narrow, scarcely 1/10 greatest eye diameter.

Arista with about 5 dorsal and 2–3 ventral branches in addition to the terminal fork. Anterior reclinate orbital about as long as proclinate, about ¾ length posterior reclinate, the latter closer to inner vertical than to proclinate. Postverticals convergent, rather well-developed, at least ⅔ length proclinate orbitals.

Mesonotum basically yellowish-brown, heavily pollinose, each hair and bristle arising from a darker brown spot, these partly fused to form an irregular longitudinal stripe in midline, darkest anteriorly, often roughly bifurcate posteriorly; on darkest specimens this central stripe gives the appearance of three consecutive wedge-shaped marks for which the species has been named. A less distinct stripe visible on each side, darkest above humeri. Scutellum yellowish-brown, densely pollinose, slightly darker centrally; anterior scutellars divergent. Prescutellars well-developed, as large as anterior dorsocentrals, the latter close to posterior pair. Acrostichals in about 10 very irregular rows. The presutural, single large humeral, 2 notopleurals and 2 sternopleurals strong. Humeri pale yellow, without spots. Pleurae pale tan, an indistinct darker brown stripe just below notopleural suture and a stronger one across middle of mesopleura and pteropleura. Two small propleural hairs. Anterior sternopleural ⅗ length posterior one. Halteres pale. Legs, including all covae and prosternum, pale yellow except for small black "knees" apically on mid- and hind-femora and basally on these tibiae.

Abdomen yellow, each tergite except the first with a broad apical dark brown band; on segments 3, 4 and 5 a similar band extends in the midline to the base of the previous segment and the transverse bands expand broadly at the angle of the tergites forming solid lateral areas.

Wings with diffuse brown clouds over apices of 1st, 2nd, and 3rd veins as well as over both crossveins; the marginal cell is faintly clouded just beyond the distal costal break, becomes gradually darker distally, culminating in the broad, dense cloud at apex of 2nd vein. Distal costal break with two terminal bristles, the dorsal one longer; 3rd costal section with heavy bristles on all but its distal ⅙; the "thorns" on underside of this section much smaller than is usual in the genus. 3rd and 4th veins parallel at end. Costa weak beyond 3rd vein but visible to 4th. Discal and 2nd basal cells separated by a weak crossvein. Costal index about 2.7; 4th vein index about 0.8. Length body, 4 to 5 mm. (in pinned specimen); wings, about 5 mm.

Distribution and types.—This species is known at present by 33 individuals collected by the writer from 7 localities in New Mexico and Arizona. All are being considered types. **Holotype,** ♂, No. 2161.8, from Horse Thief Basin Recreation Area, Prescott National Forest, about 25 miles, airline, south of Prescott, Ariz., collected June 18, 1951 by the writer. *Paratypes* as follows: Arizona: Horse Thief Basin (5), Tonto Creek, Payson (5), Mogollon Rim Road (11), Rustler Park, Chiricahua Mts. (3), Mt. Graham, Safford (1); New Mexico. Whitewater Campground, Glenwood (6), Cherry Creek Campground, Silver City (1). One paratype, from Horse Thief Basin, Ariz., is being deposited in the U. S. National Museum.

Leucophenga guttata, sp. nov.

External characters of imagines.

♂, ♀. Arista with 5 or 6 branches above and none below, the main axis not clearly bifurcate at tip; antennae pale tan, 3rd joint no darker. Front tan, dully pollinose, the orbits paler pollinose. Face, cheeks, palpi and proboscis yellowish tan, the face flat. One pair of strong vibrissae followed by a sparse row of small hairs. Cheeks very narrow, scarcely 1/10 greatest eye diameter. The two anterior orbitals about ¾ length posterior reclinate. Ocellars, verticals and postverticals strong.

Disc of mesonotum tan, heavily pale pollinose, each hair and bristle arising from a tiny brown spot, these not pollinose; scutellum entirely tan, pollinose. Acrostichals in about 10 very irregular rows; prescutellars about as long as anterior dorsocentrals. Anterior scutellars divergent. Pleurae mostly tan, weakly darker across meso- and pteropleurae, these also densely pollinose. One strong humeral; two strong sternopleurals of about equal length. Legs uniformly pale tan.

Abdomen mostly pale tan with dense gray pollinosity, with faint darker markings mainly in the form of a slender longitudinal stripe in midline and dark lateral margins. On darker specimens the apical 3–4 tergites show darker apical bands, their width about equal to that of the median stripe.

Wings marked with dark clouds in costal cell and over apex of 1st vein, over apical ¾ of marginal cell and spreading across 2nd vein into submarginal cell, around apex of 3rd vein, and over both crossveins; wing blade dusky. Costa visible between 3rd vein and 4th vein. Costal index about 2.5; 4th vein index about 1.6; 5X index about 1.0.

Distribution and types.—This species is known at present from eight individuals collected by the writer in mountains in Arizona. **Holotype,** ♂, No. 2155.18, from Rustler Park, Chiricahua Mts., Arizona, captured June 12, 1951. *Paratypes* as follows: 3, with the same data as the holotype; 4, from Kehl Springs Campground, Mogollon Rim Road near Payson, Ariz.

Leucophenga montana, sp. nov.

External characters of imagines.

♂, ♀. Arista with about 6 branches above and 3–4 below, basal to the small terminal fork. Front pollinose tan, the orbits paler pollinose. An-

tennae, face and cheeks tan, palpi and proboscis yellowish; face flat. Cheeks very narrow, scarcely 1/10 greatest eye diameter. Vibrissae strong, 2nd oral usually about ⅓ but variable in the present series. Anterior reclinate orbital about ¾ length proclinate, ⅔ length posterior reclinate, its base twice as far from the latter as from the proclinate; ocellars and verticals strong, postverticals well developed, nearly as long as middle orbital; usually with a small hair between the two reclinates of each side.

Acrostichals in about 12 very irregular rows; prescutellars strong, equal to or longer than anterior dorsocentrals. Anterior scutellars divergent. Disc of mesonotum and scutellum yellowish tan, the hairs and bristles black. Pleurae tannish yellow, as are all legs. One humeral, one presutural, two notopleurals, two sternopleurals, these nearly equal in length. Halteres pale yellow.

Abdomen tan with darker discoloration, variable in intensity and not forming any pattern, the only consistent marking being black areas on the lateral angles of the 1st complete tergite.

Wings of darkest individuals with clouds over apex of 1st vein and in costal cell, extended posteriorly, in apical ⅔ of marginal cell and continued well into submarginal cell, at apex of 3rd vein and over both crossveins. On paler specimens (perhaps tenerals) the cloud of the marginal cell is limited to the apex of the 2nd vein. Costa visible between apices of 3rd and 4th veins. Costal index 2.5–2.8; 4th vein index about 1.4; 5X index 0.7–0.9.

Distribution and types.—This is apparently a species of Northwest. Our specimens have come from California and Oregon. **Holotype,** ♂, No. 2194.6, from Shevlin City Park, Bend, Oregon, collected by the writer in August, 1951. *Paratypes* as follows: Oregon: 13, with the same data as the holotype; California: Aspen Valley, near the edge of Yosemite National Park, July, 1951 (5). Two individuals from Lake Tahoe, Calif., June, 1948, were probably also of this species but were misidentified as *Rhinoleucophenga.* They are discussed under that genus.

MICRODROSOPHILA Malloch

1921. Ent. News, 32:312.

Genotype: *Drosophila quadrata* Sturtevant.

As indicated by Sturtevant (1942), *Incisurifrons* Duda is a synonym of this genus. It is a small genus, only three species being known certainly to belong here. The diverging posterior scutellar bristles are quite distinct on the genotype but the character is not known for the other species.

It should be pointed out that *Hopkinsomyia* Malloch (1934b) is very likely the same as this genus, and its genotype, *H. convergens* Malloch, from Samoa, is, from the description, not strikingly different from *quadrata,* discussed below.

Microdrosophila quadrata (Sturtevant).

Drosophila quadrata Sturtevant, 1916. Ann. Ent. Soc. Amer., 9:341.

The types came from Alabama, and the species is known from Ga., Fla., Miss., Ill., Ind., and Texas. The species comes to banana-baited traps rather rarely and hence is uncommon in collections.

The writer captured 8 individuals by sweeping at the Aldrich Farm, Austin, Texas in Oct. and Nov., 1950. These were kept alive for some time in the laboratory. Females laid a large number of eggs, mostly deposited on the glass sides of the vial rather than on the food surface, but the larvae all died shortly after hatching. The eggs possess two terminal filaments, quite thin and about ⅔ the length of the egg itself, but these filaments, unlike those of any other species reported, fuse a short distance beyond the egg tip and appear as a single filament beyond this point. No amount of pressure on a cover-glass preparation could separate the filaments although, under high power, the two tips were visible separately, one distinctly shorter than the other. The follicle cell markings were exceptionally pronounced.

A dissection of a female revealed the following: spermathecae with simple tubular non-sclerotized centers, the parovaria quite similar in appearance but a bit smaller. Ventral receptacle tightly wound back and forth as in *Hirtodrosophila,* with about 6–7 transverse loops. Sperm were visible in the latter but not in the spermathecae.

MYCODROSOPHILA Oldenberg

1914. Arch. Naturg., 80 A 2:4.

Genotype: *M. poecilogastra* (Loew).

= *Paramycodrosophila* Duda, 1925. Ann. Mus. Nat. Hung., 22:225. New Syn.

Duda (1925) described two species from Costa Rica under the generic name *Paramycodrosophila: P. costaricana* (p. 225)) and *poeciloptera* (p. 226). Nowhere was this stated to be a new genus but he later (1927) indicated that he had erected such a genus so that the above citation must serve as generic reference. In 1927 he stated that this genus differed from *Mycodrosophila* in possessing two pairs of dorsocentrals (rather than one) and a dull mesonotum with some degree of longitudinal stripes (rather than a uniformly shining black surface). However, examples are available which illustrate every type of intermediate condition between them so that it seems best to include all of the species concerned in *Mycodrosophila.**

Members of the genus *Mycodrosophila* are fungus forms so far as known and are frequently taken on shelf fungi of the *Polyporus* type. *M. dimidiata,* the common species in the United States, can be raised on laboratory food without great difficulty.

**Paramycodrosophila poeciloptera* Duda (1925, p. 226) from Costa Rica is, from the description, clearly not a member of this genus. His account, including a figure of the wing, agrees in all essential details with the description of *Drosophila schildi* Malloch (1924a, p. 10), also from Costa Rica, a species related to *D. calloptera* Schiner.

The following key includes all of the species known from North America including the islands of the Caribbean since species from the latter area will very likely also be found in Mexico.

Key to the North American species of Mycodrosophila

1. Both crossveins heavily clouded; all femora, mid- and hind-tibiae dark brown; acrostichal hairs in 8 irregular rows; size up to 4 mm. ..... [*Paramycodrosophila punctipennis* Duda. Costa Rica. Genus very doubtful].

   Crossveins not clouded; legs pale yellow or with at most faint rings on femora; acrostichal hairs in 6 rows; size below 3 mm. 2

2. Mesonotum light brown or yellowish brown with two darker stripes between or on dorsocentral rows 3

   Mesonotum unicolorous, more or less shining black to brown, without noticeably darker longitudinal stripes 4

3. Mesonotum light brown with 2 darker stripes just within dorsocentral rows; lower pleurae and all legs pale yellow; abdomen shining brown with some yellowish areas visible basally near angle of tergites ..... *mexicana* (Whlr.)

   Mesonotum light yellow with 2 brown converging stripes; brownish stripes just above humeri, along notopleural suture and across sternopleura; legs pale yellow, femora sometimes with darker median bands; abdominal tergites with complex pattern of yellowish areas ..... [*Paramycodrosophila costaricana* Duda. Costa Rica].

4. Wings with blackish color at costal break continued below as a broad band reaching anal cell; small dark clouds around apices of 2nd and 3rd veins, less distinct cloud on 4th vein ..... [*M. projectans* (Stvt.) West Indies, Haiti].

   Wings lacking the described band, with at most a small diffuse darkening near base of marginal cell 5

5. Anterior dorsocentrals undeveloped; scutellum with thick, velvety black pile ..... *dimidiata* (Loew).

   Anterior dorsocentrals more or less developed, distinctly larger than the surrounding acrostichal hairs; scutellum shining or only faintly pollinose 6

6. Abdominal tergites mostly black, yellow areas present only on 5th and 6th segments, but variable; wings nearly clear; mesonotum light brown, scutellum darker, front lighter ..... [*M. pleuralis* (Williston). West Indies].

   Some yellow areas on all abdominal tergites except apical one; wings with a faint diffuse brown area in marginal cell just below distal costal break; mesonotum, scutellum and front dark chestnut brown ..... [*M. thoracis* (Williston). West Indies].

Mycodrosophila dimidiata (Loew).

Drosophila dimidiata Lw., 1862. Berlin. ent. Zeit., 6:230 (Cent. II. No. 95).

This is a widely distributed species over most of the United States, our most western records being from Colorado. It has been recorded from most of the states east of the Rocky Mountains. It is attracted to banana-baited traps to only a small degree but can be reared on standard laboratory food rather easily.

Mycodrosophila mexicana (Wheeler).

Paramycodrosophila mexicana Whlr., 1949. Univ. Tex. Publ. 4920:164.

This species is known only from the flies originally collected by Dr. G. B. Mainland from a bracket fungus on a willow tree near Jacona, Michoacan, Mexico in August, 1942.

NEOTANYGASTRELLA Duda

1925. Ann. hist.-nat. Mus. hung., 22:201, 203.

Genotype: *N. tricoloripes* Duda.

This genus was recently reviewed by Frota-Pessoa and Wheeler (1951). One species is known from Mexico but two species from Costa Rica, not definitely assigned to this genus, may belong here (*Drosophila bicoloripes* Malloch, *D. nigricosta* Malloch).

Neotanygastrella leucopoda (Wheeler).

Chymomyza leucopoda Wheeler, 1949. U. Tex. Publ. 4920:161.

The only specimen of this species was taken by the writer near Morelia, Michoacan, Mexico in August, 1947. A related species, *N. brasiliensis* (Frota-Pessoa) has been reared from larvae found in decaying fruit of *Artocarpus integrifolia* (Moraceae) in Brazil.

PSEUDIASTATA Coquillett

1908. Proc. Ent. Soc. Wash., 9:148.

Genotype: *P. nebulosa* Coq.

This genus has recently been reviewed by Sabrosky (1951) who summarizes the generic characters and gives figures of the male genitalia for several species. He points out that the anterior frontal bristles are convergent, suggestive of the Milichiidae, and the genus may run to that family in some keys.

Although information concerning the larval habits of the type species is lacking, larvae of other species are predacious on the pineapple mealybug, *Pseudococcus brevipes*. According to Quayle (1938) this mealybug occurs primarily on pineapples and bananas in the tropical regions of both hemispheres, and also on sugar cane in both Florida and Louisiana.

Sabrosky states that the adults of the various species are virtually indistinguishable on superficial characters, the striking wing pattern being too variable for diagnosis. The following key to the two species known to occur in North America is taken from his account of the visible male genitalia.

Key to the Nearctic species of Pseudiastata

1. Males with 9th tergite strongly produced ventrad on each side as a genital forceps, each lobe obviously longer than broad and not tapering, the sides approximately parallel up to the somewhat rounded apex; in anterior aspect, flanking the midline, are two thumb-like processes, each of which bears three distinct bristles ..... *nebulosa* Coq.

   Males with the 9th tergite strongly produced below on each side, the genital forceps so formed being approximately as broad as long, not tapering, and distinctly subtruncate; in anterior aspect, the two processes flanking the midline are relatively short and acute, without bristles ..... *pseudococcivora* Sabr.

Pseudiastata nebulosa Coquillett.

P. nebulosa, Coq., 1908. Proc. Ent. Soc. Wash., 9:148.

The type was taken at a light on Plummer's Island, Maryland. Sabrosky *(op. cit.)* has identified a specimen from Perry, Georgia as probably belonging to this species.

Pseudiastata pseudococcivora Sabrosky.

P. pseudococcivora Sabr., 1951. Bull. Ent. Res., 41:624.

This species was described from ten specimens from Panama Canal Zone and one intercepted in quarantine at Laredo, Texas entering from Mexico. Sabrosky believes that this is the species which was introduced into the Hawaiian Islands for the control of the pineapple mealybug.

RHINOLEUCOPHENGA Hendel

1917. Deutsche. ent. Zeit., Berlin, 1917:44.

Genotype: *R. obesa* (Loew).

Hendel established the genus for his new species *pallida* from Peru. Loew described *Drosophila obesa* from Texas and this species was redescribed by Johnson in 1913 as *Phortica hirtifrons* from Florida. Later, Sturtevant (1918) established the genus *Pseudophortica* for *obesa* and placed *hirtifrons* as a synonym. Malloch and McAtee first stated that *pallida* was also a synonym of *obesa* and placed *Pseudophortica* as a synonym of *Rhinoleucophenga.*

Whether *pallida* Hendel and *obesa* Loew are the same species is still open to question. Duda (1927), having compared a specimen of *pallida* determined by Hendel, concluded that the two were distinct. In a more recent study of the genus, Malogolowkin (1946) concluded that they were the same, recorded *obesa* from Brazil, and figured the wing and male genitalia of the Brazilian form. Using specimens from Nevada and Colorado, Hsu (1949) examined the male genitalia and found considerable difference between these specimens and the figure of Malogolowkin *(op. cit.)*. He concluded that since *obesa* was described from Texas, the Brazilian form could not be *obesa*. However, we have reason to think that the specimens used by Hsu were misidentified; among our preserved material we have found two specimens from Lake Tahoe, California identified by the field collectors as *obesa* but which, on closer examination, prove to be referable to *Leucophenga montana,* n.sp., described earlier in this article. Further, the male genitalia of the specimen figured by Hsu (Pl. II, fig. 4) are remarkably similar to that of *L. pulcherrima* (Pl. III, fig. 1). Finally, we have since captured a large number of specimens, presumably of *obesa,* in Texas, and these show the tooth-bearing clasper as do the flies from Brazil. We have not yet established that the Texas and Brazilian species are the same, but at present it seems probable. However, should it be shown that the two are distinct, then it is likely that *pallida* would be the genotype.

Rhinoleucophenga obesa (Loew).

Drosophila obesa Loew, 1872. Berl. ent. Zeit., 16:102 (Cent. X, No. 85).

The species was described from specimens collected in Texas by Belfrage. We have taken specimens sporadically in central Texas, but our largest collections have been made in Limpia Canyon, Davis Mts., Texas, where, for example, we captured 42 individuals at traps in June, 1951. However, these specimens have a distinctly darker abdomen than others we have seen and may represent a new species. *R. obesa* seems to be widspread over the southeastern states, from Va. and Fla. to Okla. and Texas. Patterson (1943) records 20 specimens from Michoacan, Mexico as an undetermined species of the genus; this was a misidentification, and the species was later (Patterson and Mainland, 1944) described as *Leucophenga paludicola.*

We were able to secure a few eggs of the form from the Davis Mts., Texas, and reared these through to adults. The eggs were laid on the glass sides of the containers; their structure was remarkably similar to that of *Sinophthalmus pictus* (fig. 1), but were white rather than pale brown.

Costa Lima (1935) reports that the Brazilian *R. obesa* lives as a parasite on the coccid *Aclerta campinensis.* It is tempting to postulate some correlation here between the parasitic mode of life and the type of egg known to be present in *S. pictus,* in our *obesa,* and at least some species of *Gitona* (which also has parasitic members). As is described later, *S. pictus* has habits of egg and larval deposition which make it seem possible, at least, that it has parasitic larvae; larvae of *obesa* from this country have not been found nor have those of *Gitona bivisualis.* The possibility of parasitic larvae in these forms should be carefully looked for.

SCAPTOMYZA Hardy

1849. Proc. Berwickshire Naturalists Club:349.

Genotype: *S. graminum* (Fallen).

Most of the species of this genus have leaf-mining larvae although there are apparently some exceptions. As a whole, they are attracted to banana bait only slightly but general sweeping in grass and weeds of various sorts will often turn up certain species in enormous numbers. We have found that many species can be raised in the laboratory if non-yeasted food is used; some species, however, will not deposit eggs on standard laboratory food.

The genus was established by Hardy for *Drosophila graminum* Fallén and *D. flaveola* Meigen, and the first of these was selected as type in 1910 by Coquillett. Since then, over fifty species and varieties have been described by various workers over the world, and the resulting confusion is scarcely equalled in any other genus. It is becoming apparent that many specific names are synonyms, many so-called varieties are good species, a number of species are referable to other genera, and, finally, that much past work on the genus is wholly unreliable. Dr. O. Duda at one time con-

sidered the genus tenable at most as a subgenus of *Drosophila* but at the same time propósed dividing it into two subgenera: *Parascaptomyza,* for the forms with two acrostichal rows (considered by him as all being color variants of *graminum*), and subgenus *Scaptomyza* Hardy, for those forms with four acrostichal rows. Hendel (1928) pointed out, however, that the typical subgenus, *Scaptomyza* Hardy, must contain *graminum,* and that *Parascaptomyza* Duda must become a synonym of that name. Hendel then established a new genus, *Scaptomyzella* (misprinted *Scaptomyzetta*), for the species with four acrostichal rows, and placed *Scaptomyza* Duda nec Hardy as a synonym. Malloch (1932) argued that one genus might well contain all of these forms since, on the basis of the American species, *adusta* Loew would scarcely fit either of the proposed divisions, and *vittata* Coquillett would certainly deserve still another subdivision.

The writer is of the opinion that we know far too little about the genus to warrant erection of subgenera. There are, however, a number of natural groups which can be recognized but these are probably best considered as species groups. As in *Drosophila* there are a number of species which do not logically belong in any such group and I have refrained from establishing monotypic groups for such species except for *S. graminum* which is quite well known.

The following species groups may be recognized:

1. graminum species group

Only member, *S. graminum.*

Blackish to yellowish species; 2 acrostichal rows; long apical scutellar bristles; one strong humeral; one ventral branch on arista basal to terminal fork; no wing spot; pale palpi; small parovaria. This species is easily reared in the laboratory.

2. adusta species group

Contains *S. adusta, paradusta* n.sp., and possibly *hirsuta.*

Yellowish to light brownish species; 4 acrostichal rows; apical scutellars short, bent upright; one humeral; 2 ventral branches on arista; with apical wing spot (only slightly indicated on *hirsuta*) ; pale palpi; small parovaria. *S. adusta* is easily reared; *paradusta* has not been reared; *hirsuta* is raised with difficulty.

3. vittata species group

Contains *S. vittata, paravittata* n.sp., species A, as well as *fuscinervis* and *nigripalpis,* two South American species described by Malloch.

Pale yellowish species with some longitudinal stripes; 2 acrostichal rows; apical scutellars short, bent upright; one humeral; 2–3 ventral branches on arista; no wing spot; blackish palpi; small parovaria. Species A is easily reared; *paravittata* has not been raised; *vittata* is unknown for this character.

4. terminalis species group

Contains *S. terminalis,* the undescribed species C, D and E, as well as *unipunctum* (Europe), *maculifera* (South America), etc.

Blackish species; 4 acrostichal rows; long apical scutellars; 2 humerals; 1–2 ventral branches on arista; with apical wing spot; palpi pale; small parovaria. As far as known, all species can be raised rather easily.

5. montana species group

Contains *S. montana, nigrocella, borealis* n.sp., *nigrita* n.sp., as well as *tetrasticha* (Europe), etc.

Blackish or yellowish species; 4 acrostichal rows; long apical scutellars; 2 humerals; one ventral branch on arista; no wing spot; palpi pale; parovaria very large, 2–6 times size of spermathecae; ovipositor large, blunt apically, with large, coarse teeth; male anal plate elongate dorso-ventrally, protruding below. None of these species has been reared in the laboratory.

Key to the Nearctic species of Scaptomyza

1. Acrostichals in 2 rows; 1 prominent humeral; wings without an apical spot 2
   Acrostichals in 4 rows anterior to dorsocentrals; 1 or 2 humerals; wings with or without apical spot 5
2. Palpi yellow; apical and basal scutellars about equal in length; arista with 1 branch below basal to fork ..... *graminum* (Fall.)
   Palpi dark; apical scutellars about half length basals and bent strongly upright; arista with 2–3 ventral branches 3
3. Mesonotal stripes prominent; ocellar darkening continued forward in a line to antennal bases; presutural dorsocentrals but little larger than other acrostichals ..... *paravittata* n. sp.
   Mesonotal stripes weak; ocellar darkening limited to the triangle; presutural dorsocentrals rather well developed 4
4. Two nearly equal oral bristles on each side, their length about equal to length of proclinate orbital; presutural dorsocentral as long as anterior notopleural; terminal tergites quite dark, lateral areas of other tergites often much lighter; ♂ genitalia with about 3 conspicuous black spines on each side ..... *vittata* Coq.
   One pair of long orals, nearly twice length proclinate, 2nd oral barely half length 1st; presutural dorsocentral no stronger than humeral; lateral areas of tergites usually about as dark as terminal tergites; ♂ genitalia without spines ..... *Species A*
5. Wings with a dark cloud at apex of 3rd vein (but may be weak and scarcely noticeable on tenerals) 6
   Wings completely lacking an apical cloud 9
6. Apical scutellars about half length basals, bent strongly upright; 1 strong humeral; general color tannish, abdomen darker 7
   Apical scutellars longer, directed posteriorly, their tips reaching about as far behind as those of basals; 2 strong humerals; brownish to blackish species ..... *terminalis* Lw., *Species C, D & E.*
7. Mesonotum mostly brown; apical wing spot small and indistinct ..... *hirsuta* Whlr.
   Mesonotum yellowish to pale brownish; wing spot distinct 8
8. Scutellum and disc of mesonotum nearly unicolorous; arista with 4–5 dorsal branches basal to fork; palpi with very stout black bristles; ♂ anal plate with sparse thin hair ..... *adusta* (Loew).
   Scutellum distinctly darker than mesonotum; arista with about 3 dorsal branches; palpal bristles not exceptionally stout; ♂ anal plate densely covered with long hairs; posterior crossvein often with a slight cloud ..... *paradusta* n. sp.

9. Abdomen entirely yellow, noticeably contrasting with the gray pollinose metanotum; scutellum gray centrally, the lateral margins yellow ..... *Species F*

Not colored as above, the abdomen as dark as or darker than thorax, or both pale yellow 10

10. Posterior orbits with 1 or 2 extra orbital hairs between posterior reclinate orbital and inner vertical; ♂ anal plate noticeably elongate dorso-ventrally, protruding below; ♀ ovipositor large, blunt, with coarse teeth 11

No extra orbital hairs as described; ♂ anal plate and ♀ ovipositor as described or not 12

11. Mesonotum, pleurae and abdomen dark gray to brown ..... *montana* Whlr.

Mesonotum and pleurae pale yellow to tannish yellow, the abdomen the same or a bit darker ..... *nigrocella* Whlr.

12. Apical scutellars short, strongly bent upright; 1 strong humeral; arista with 2 ventral branches; knob of halteres gray; ♂ anal plate with an exceptionally dense, but nearly microscopic, cluster of fine hairs on ventral ¼ ..... *hirsuta* Whlr.

Apical scutellars as long as basals or at least reaching about the same distance posteriorly; 2 humerals; arista with 1–2 ventral branches; knob of halteres whitish yellow; ♂ anal plate not as described 13

13. Femora nearly black, tibiae and tarsi somewhat brownish, the fore coxae pale; mesonotum black, pollinose ..... *nigrita* n. sp.

Legs nearly uniformly pale yellow; mesonotum yellow or grayish brown with browner median stripe 14

14. Mesonotum and pleurae grayish brown to blackish brown with a faintly indicated median brown stripe; ♂ anal plate elongate dorsoventrally, protruding below ..... *borealis* n. sp.

Entirely yellow species except for the shining black abdomen; ♂ anal plate not large and protruding ..... *Species B.*

Scaptomyza graminum (Fallén).

Drosophila graminum Fall., 1823. Dipt. Suec., Geomyz. :8.

This species is widely distributed over the entire United States and has been reported from all the major areas of the world. We have examined specimens, including the ♂ genitalia, from Canada, Hawaii, Europe and Lebanon, and they agree with the form in this country. The biology of the species has been described by Frost (1924) and Stalker (1945) presents a redescription based on material from Missouri, and discusses its ecology, seasonal distribution, cytology and comparative genetics. Patterson (1943) describes the internal anatomy, eggs and puparia.

This species is exceedingly variable in color; specimens captured at identical times and localities may be quite pale, scarcely exhibiting any color pattern, while others may be nearly black. Stalker believes that the variation may be due to the season and the age of the flies. *S. graminum* may be raised in the laboratory with ease.

Scaptomyza adusta (Loew).

Drosophila adusta Lw., 1862. Berl. ent. Zeit., 6:231 (Cent. II, No. 98).

This is a very common and widely distributed species over the eastern half of the continent, extending west into the foothills of the Rocky Mountains. The larvae are undoubtedly leaf miners for the most part but apparently other situations will serve; Malloch (1915), for example, obtained larvae and puparia from sap exuding from a mulberry tree at Urbana, Ill., and the writer has reared specimens from rotting cactus at Austin, Texas. *S. adusta* can be raised in the laboratory with comparative ease. Patterson

(1943) describes and figures the eggs, puparia and internal reproductive organs.

Scaptomyza paradusta, sp. nov.

External characters of imagines.

Front pale tan, orbits lighter, granulose, ocellar triangle not distinct, blackish between ocelli. Head bristles large. Proclinate and anterior reclinate orbitals with their bases at about the same level, the latter about ⅔ as long as the proclinate and nearly ½ length of posterior reclinate. Antennae pale tan, 2nd joint with several stout bristles; arista with about 3 dorsal and 2 ventral branches in addition to the terminal fork. Face pale whitish yellow; carina low, elongate, evenly rounded, ending above line of vibrissae. Vibrissae strong, 2nd orals weaker, about ½ as long as 1st orals. Palpi whitish yellow with about 2 moderately stout bristles and several smaller ones. Cheeks whitish yellow, their width about ¼–⅕ the greatest diameter of the eyes. Eyes bright red in life, with fine, dense, pale-colored pile.

Mesonotum light reddish brown overlaid with rather heavy whitish pollen, a slightly darker stripe between median acrostichal rows; scutellum similarly darkened. Acrostichal hairs in 4 rows anterior to the dorsocentrals, 2 rows between them. Apical scutellars shorter than basal ones and carried turned upwards; basal pair convergent. One strong humeral. Anterior sternopleural thin, about ⅖ length large posterior one.

Legs uniformly pale yellow. All tibiae with preapical bristles, a distinct apical present only on 2nd. Abdominal tergites brown, pollinose except on last complete one which is shining. Male anal plate covered with dense, long hairs; female anal plate relatively densely haired. Ovipositor not prominent, not heavily toothed. Wings with faint yellowish cast. A rather large blackish cloud at apex of 3rd vein. Posterior crossvein with faint but often distinct narrow cloud. Two strong bristles at distal costal break. 3rd costal section with heavy bristles on its basal ½, these ceasing just before the apical cloud. Costal index 3.3–3.4; 4th vein index about 1.4; 5x index about 1.25.

Length body, ♂ : 2.5 mm.; wings: 2.6 mm. (in pinned specimen).

Female, body length: 2.8 mm.; wings: 2.7 mm.

Distribution and types.—Known at present from 14 specimens from California and one from Arizona. **Holotype,** ♂, No. 2175.12, from Dark Canyon Forest Camp, San Bernardino National Forest, near Mt. San Jacinto, California, taken by the writer in sweeping in July, 1951. *Paratypes:* California: 4, from the same locality as the holotype, Alma (1), Pismo Beach (1), Aspen Valley near Yosemite National Park (1), and the following in the collection of Dr. A. H. Sturtevant: Berkeley (1), Pacific Grove (3), Charleton Flats near Pasadena (2); Arizona: Oak Creek Canyon south of Flagstaff (1).

Notes.—All of the above specimens were taken by sweeping and did not seem to be attracted to traps. We have not succeeded in getting females

to deposit eggs in the laboratory. This species simulates members of the genus *Chymomyza* in several respects, as does *S. hirsuta*. Males of these species have a large whitish triangular area medianly on the pregenital tergite as do many species of *Chymomyza*. More remarkable, however, is the habit of *paradusta* to wave the wings and spar with one another using the fore legs.

Scaptomyza hirsuta Wheeler.

S. hirsuta Whlr., 1949. Univ. Tex. Publ. 4920:166.

Described from six specimens from Puebla, Mexico, we have since taken about 25 individuals by sweeping over *Rumex* sp. at Rustler Park, Chiricahua Mts., Ariz. A stock was established from these specimens but was lost by accident. In this species the front tibiae and tarsi are darkened and the flies wave their wings like *Chymomyza*. It is probably related to *paradusta*, described above.

Internal characters.—Testes of aged male deep yellow, with about 1½ large outer coils and a short, inner half-coil; paragonia large, U-shaped. Ejaculatory sac with two diverticula, each bifurcate near its base into two branches of unequal length, one about ⅓ the length of the other.

Spermathecae with heavily sclerotized centers, of moderate size; parovaria slender, as long as spermathecae but smaller; ventral receptacle large and thick basally, followed by a tight, tangled mass of irregular coils. The eggs have four very short filaments, and the follicle cell outline is very plain; length, about 0.5 mm.

Scaptomyza vittata (Coquillett).

Drosophila vittata Coq., 1895. *in* Johnson, 1895. Proc. Acad. Nat. Sci. Phila. :318.

Coquillett described *vittata* from a single male taken by Mrs. Slosson at Charlotte Harbor, Florida. He later reported a specimen from Porto Rico but as indicated below, this was probably of a different species.

In examining a series of specimens determined as *vittata* it became apparent that two forms were concerned. Although the original description omits a number of characters that are critical in this genus, I believe that the form given this name in the key is the one described by Coquillett. However, due to the uncertainty, the other form is not being given a new name at present, but is referred to here as species A.

From among Dr. Sturtevant's specimens I was able to determine that the following localities were represented by true *vittata:* Kushla, Ala., Lakeland, Fla., Cuba and Costa Rica. Our laboratory has specimens from Miss., and, until recently, a living stock from Macon, Ga., collected by Dr. H. Stalker. The stock was not difficult to maintain, but was lost through accidental neglect. It was used to prepare the following description of internal characters.

Internal characters of imagines.

Testes with two thick, pale yellow outer coils, the inner portion lemon yellow, not distinctly coiled. Two ejaculatory sac diverticula of moderate length, each bifurcate at its apical third into branches of unequal length.

Spermathecae with oval, moderately sclerotized centers; parovaria round, about half size of former. Ventral receptacle very loosely and irregularly coiled, with an average of 10–15 coils.

The figure of the ♂ genitalia given by Hsu (1949. Pl. III, fig. 6) is apparently referable to the following form, species A.

Scaptomyza species A.

As stated above, under *vittata,* specimens of this unnamed species have been confused with that one. Localities for this form represented among Dr. Sturtevant's specimens are as follows: Arlington, Va., Greenville, S.C., Kushla, Ala., and Costa Rica. A male specimen from Mexico was used by Hsu (1949) to illustrate the ♂ genitalia of *vittata.* That specimen came from Puebla, and we have single individuals from Vera Cruz and the Federal District. This is very likely the form reported by Malloch and McAtee (1924) from Va. and Md., and may have been the one reported by Coquillett (1900) from Porto Rico since Sturtevant (1921) states that he examined the specimen and was unable to convince himself that it was the same as *vittata.*

Scaptomyza paravittata, sp. nov.

External characters of imagines.

♂, ♀. Arista with about 8 branches, two below in addition to the terminal fork. Antennae pale tannish yellow; 2nd section with 2 large bristles and several smaller ones. Third antennal segment small. Front pale tan with a narrow central blackish stripe from postverticals across the ocellar triangle to base of antennae. Proclinate and anterior reclinate orbitals with their bases at about the same level; anterior reclinate about half length proclinate, ⅓ length posterior reclinate. Vibrissae well-developed, followed by an irregular series of fine hairs. Palpi black from near the base, with a strong bristle distally. Face, clypeus, cheeks and proboscis pale tannish yellow. Center of face lowly convex, without a distinct carina. Cheek width slightly less than half greatest diameter of eyes, the latter deep red.

Acrostichal hairs in two regular rows both between and in front of the dorsocentrals; no prescutellars. An additional presutural pair of dorsocentrals in line with the usual ones and separated from the median pair by a single hair; length of this anterior dorsocentral about ⅓ length median ones and diverging outward. Median dorsocentrals about as far forward of the posterior ones as the latter are from each other. One prominent humeral. Two prominent sternopleurals, the anterior one thinner and about ⅔ length posterior. Apical scutellar bristles about half length basal pair, bent upright and cruciate. Basal scutellars parallel or slightly divergent. Mesonotum pale tan with three longitudinal dark brown stripes as follows: a central stripe from anterior edge to scutellar apex, exactly delimiting the acrostichal rows; a lateral stripe on each side originating just above the humerus and continuing to just above wing base. Pleurae pale tan with a longitudinal stripe originating just below humerus and

continuing across base of halteres causing the two basal segments of this structure to be darkened, the terminal portion being pale. Pleurae below this stripe and all legs pale yellow. Preapicals on all tibiae, apicals evident only on 2nd tibiae. Males with a series of semi-erect hairs on fore tarsi, their length equal to or slightly less than thickness of tarsus.

Abdomen pale yellow with black bands as follows: on the first three tergites the bands appear only as discolored spots on either side of the midline but are present as solid black lateral areas; on remaining tergites the bands are distinct from either side of the median interruption, expanding broadly at the angle and becoming progressively larger and darker posteriorly. The male abdomen is less distinctly banded than the female. Ovipositor plates quite pale, only faintly chitinized, and bearing a few slender hairs and bristles; the plates are bluntly rounded posteriorly.

Wings clear, veins pale brownish. Two strong bristles at distal costal break. Third costal section with heavy bristles on its basal ¼. Costal index about 2.9–3.0; 4th vein index about 1.5; 5x index about 1.8.

Length body, male: 2.0 mm. (in preserved specimen), wings: 2.2 mm.; female body: 2.3 mm., wings: 2.6 mm.

Internal characters of imagines.

Testes pale yellow with 2 large, pale outer coils, 2 smaller, deeper yellow inner coils leading to an inner series of 3–4 tight, very small coils which join the duct. Ejaculatory sac with two long, thick, unbranched diverticula.

Spermathecae large, non-sclerotized, without a visible central cavity, their stalks quite short and ending bluntly; parovaria also blunt-tipped, with short stalks. Ventral receptacle of medium length, twisted back and forth, not noticeably coiled. Anterior Malpighian tubes much longer than posterior, their common stalks about 1/12 their total length; posterior tubes with their ends apposed but lacking a continuous lumen, their common stalk about 1/10 their total length.

Other characteristics, relationship and distribution.

Eggs.—White, with a decided wrinkled appearance; a thickened ridge runs along the midline nearly the entire length, branching just before the end to form two short filaments. Length about 0.6 mm.

Puparia.—Pale tannish yellow, weakly chitinized. Anterior spiracular horns extremely short, each with about 5–6 short branches; posterior spiracles tightly parallel. Length: 3.0 mm.

Distribution and types.—We have taken this species in rather large numbers (several hundred) by sweeping in the Pasadena area of southern California. Most came from two localities: the Arroyo Seco of Pasadena and South Pasadena, and a marshy area near Rosemead. **Holotype**, ♂, from Rosemead, California, collected by the writer Feb., 1954. *Paratypes* as follows: California: 10, from the same locality as the holotype, Pasadena (2), South Pasadena (2), the Arboretum, Arcadia (3).

Notes.—A number of larvae and puparia have been taken from water cress *(Nasturtium officinale)* in which they were living as leaf-miners.

Pupation occurred on the leaves at the edge of the mines. All the adults collected were taken by sweeping over this plant. Females will deposit eggs readily on the standard culture medium for *Drosophila* but it has not been possible as yet to raise the larvae to maturity.

It may be of interest to record that the locality mentioned above also yielded adults of *S. terminalis, graminum* and *montana,* both larvae and puparia of the latter being also found on the water cress.

Scaptomyza terminalis (Loew).

Drosophila terminalis Lw., 1863. Berl. ent Zeit., 7:32 (Cent. III, No. 60).

The specific distinctions in the *terminalis group* are extremely confused. In the past any specimen with the combination of external characters cited earlier for the group has been called *terminalis,* a species described from Alaska. Intensive study has shown, however, that although specimens from various parts of the country (or other countries) appear to be identical in color and gross morphology, the details of the external genitalia and internal reproductive organs vary greatly. It would seem that we have in this group a situation comparable to that found in several Dipterous groups in which a number of species can be recognized by the male genitalia which otherwise had been considered a single, wide-spread species. The members of this group, fortunately, can be raised in the laboratory without great difficulty so that here we have an opportunity of testing the validity of species based solely or primarily on ♂ genitalia. This project will be started in the near future. At the present time, however, it seems best to indicate the form we believe to be true *terminalis,* to refrain from naming the other forms but to give some idea of their known distributions.

The types of *terminalis* came from Sitka, Alaska. The U.S. form most likely to be this species has been found by us along the Pacific coast, from Lower California of Mexico, the coasts of California, Oregon and Washington. Our most northern collection, from the Olympic Peninsula, Wash., is but little more than 750 miles, airline, from Sitka, and from our knowledge of the area, it seems nearly certain that the same form would continue up the coastal regions to Alaska. It should be pointed out, however, that should the above conclusion be shown to be in error, then the U.S. form should be called *apicata* Thomson, whose types came from San Francisco.

In addition to the west coast records, we have taken this species in two localities in Arizona: Oak Creek Canyon near Flagstaff and Patagonia. The characteristic features of the ♂ genitalia are: lower posterior corner of genital arch with a long pointed, chitinized "toe", usually visible on pinned specimens; clasper rather pointed below, with a row of about 8–10 strong blunt teeth; anal plate with a small cluster of about 10 thick, black bristles along lower edge, longer, thinner and more pointed than the teeth of the clasper.

Internal characters of imagines.

Testes of aged male bright lemon yellow with about 1½ large coils arranged like a corkscrew, no inner coils. Paragonia weakly S-shaped,

intertwined with testes. Ejaculatory sac with two short, thick diverticula, each bifurcate at about its middle with a long and a short branch.

Spermathecae with small sclerotized centers and thick stalks; parovaria not more than half the size of the former. Ventral receptacle a weakly bent tube basally, a tangled mass apically, without distinct coils.

Notes.—This species was rather common around Pasadena, Calif., where the writer has reared it from water cress *(Nasturtium officinale)*.

Scaptomyza species C.

This is the form used by Hsu (1949) to illustrate the ♂ genitalia of *terminalis*. That specimen came from Caliente, Nevada. The genital arch of the male lacks the "toe", and the clasper is more truncate with the teeth arranged in two groups, 2–3 in the upper group, 9–10 in the lower group. We have no other records of this form.

Scaptomyza species D.

This form is known to us by several stocks taken in the mountains near Silver City, New Mexico, as well as a few specimens from Rustler Park, Chiricahua Mts., Ariz. The ♂ genitalia may be briefly characterized as follows: genital arch with a short blunt "toe", very weakly chitinized and not usually visible externally on pinned specimens, and with a dense cluster of long hairs just below the projection; clasper semi-elliptical, with a row of about 6 black teeth, rather long and slender and spaced rather far apart, plus a few pale bristles apically; anal plate without unusual characters.

Scaptomyza species E.

This is largely a hypothetical form; there are in the literature numerous references to *terminalis* from the New England states, and, on the basis of our experience, this may well represent still another form in the series. Unfortunately, we have not had specimens of such a form available for study.

Scaptomyza montana Wheeler.

S. montana Whlr., 1949. Univ. Tex. Publ. 4920:166.

Originally described from Glacier National Park, Montana, the writer has since found this species to be rather common in southern California where specimens were reared from larvae and pupae found on water cress *(Nasturtium officinale)*. We have not, however, succeeded in raising the species in the laboratory. This is unfortunate for, as discussed below, completely yellow specimens are taken occasionally which may represent another species or may be nothing more than a yellow mutant of *montana*. There seems to be no way to settle this problem except by breeding experiments. In addition to the many individuals taken in the Pasadena area of California, we have taken this species near Crescent City, Calif., and along the Rogue River near Gold Beach, Oregon, and have examined specimens in Dr. Sturtevant's collection from Vashon, Washington, and from Palo Alto, Pacific Grove and Stanford University campus, California.

Internal characters of imagines.

Testes of aged male yellowish-orange, darkest basally, sac-like, not coiled. Paragonia S-shaped, longer than testes. Ejaculatory sac with two very long posterior diverticula each bifurcate shortly beyond its base and one or both of these branches again bifurcate shortly before its end, these latter bifurcations variable among different specimens; the right and left series of branches form a tangled mass on either side.

Spermathecae with oval, heavily sclerotized centers and thick stalks; parovaria round to oval, very large and balloon-like, six to eight times the size of the spermathecae, their stalks alone about as long as the total length of the spermathecae. Ventral receptacle rather short and folded back on itself about 2½ times in the manner of the *Hirtodrosophila*.

Scaptomyza nigrocella Wheeler.

S. nigrocella Whlr., 1949. Univ. Tex. Publ. 4920:167.

The specific status of this form is open to considerable doubt. It was described from a few specimens from Jasper, New York. The external male genitalia described by Hsu (1949) were quite similar to *montana*. More recently we have taken a number of specimens in California, along with many typical *montana*, and have compared these two color forms in every way possible, with the result that, except for the extreme color difference, they seem to be identical. Further, a re-examination of the New York specimens indicates that they are also the same as the western material. The situation is further complicated by the fact that in certain localities in northern California, as well as our extensive collection efforts at Lake Wenatchee, Wash., and Hood River, Ore., only the pale yellow form could be found. Further, I know of no record of the dark form from the eastern United States. It is a very intriguing problem which deserves continued study.

There is the possibility that this is the same as some European form, since pale yellow forms have been reported from there on numerous occasions. The names used most frequently for the European form are *flava* Fallén and *flaveola* Meigen. Collin (1911) who has examined Fallén's types, states that *flava* is a true *Drosophila*, and, finally, there seems to be considerable diversity of opinion as to just what *flaveola* Meigen really is. In view of all the foregoing, it seems impossible to settle the questions raised by these yellow specimens at present.

Scaptomyza borealis, sp. nov.

External characters of imagines.

♂, ♀. Front pale tan, ocellar triangle and posterior orbits dark gray pollinose, anterior orbits and front yellowish. Proclinate and anterior reclinate orbital situated at about the same level or the reclinate farther forward, the latter about ½ length the other orbitals. No extra orbital hairs between posterior reclinate and inner vertical. Antennae pale yellow throughout. Arista with about 7 branches, only 1 below in addition to the terminal fork. 2nd antennal segment with 2–3 stout hairs. Face pale

whitish yellow, carina an exceedingly low ridge, not nose-like. Vibrissae strongly developed, the following orals thin, about ¼ length 1st. Palpi pale yellow with about 2 strong apical bristles. Cheeks pale whitish yellow, their width about ⅕ greatest diameter of eyes, these dark red with light-colored pile.

Acrostichal hairs in 4 rows in front of the dorsocentrals, in 2 rows between them; no prescutellars. Apical scutellars about ⅔ length basal ones, reaching about the same distance posteriorly. Two humerals; posterior sternopleural large, anterior one thin, about ½ as long. Mesonotum and scutellum pale grayish brown, dull, pollinose, with a brownish narrow stripe between median acrostichal rows. Mesopleura and pteropleura similarly grayish brown, pollinose, sternopleura and hypopleura much more yellowish. Halteres pale. Legs pale yellowish, with the usual bristles.

Abdomen uniformly brown, faintly shining. Male anal plate large, protruding below; ovipositor plates large, yellow, with coarse marginal teeth. Anal plates of both sexes with long hair.

Wings clear, without an apical cloud. Two strong bristles at distal costal break; 3rd costal section with heavy spinules on slightly less than its basal half. Costal index about 3.5; 4th vein index about 1.3–1.4; 5x index about 1.8.

Length body, male (in pinned specimen), 2.5 mm.; wings, 2.8 mm. Female slightly larger than male.

Distribution and types.—This is probably a fairly common species across the northern portion of the United States, but we have seen relatively few specimens. **Holotype,** ♂, from Ossipee, New Hampshire, Aug. 4, 1950 (A. H. Sturtevant). *Paratypes* as follows: New Hampshire: Hanover (1, Sturtevant coll.); Massachusetts: Fall River (1, Stvt. coll.), Westport Factory (1); Vermont: Norwich (1, Stvt. coll.); Michigan: Detroit (1, G. Steyskal, in collection of H. Stalker).

Scaptomyza nigrita, sp. nov.

External characters of imagines.

♂, ♀. Front yellowish orange, more yellowish anteriorly, the large ocellar triangle and broad orbits blackish with gray pollinosity. Anterior reclinate orbital placed slightly in front of the proclinate and about ⅓ its length, the posterior reclinate slightly longer and stronger than proclinate; not extra orbital hairs between posterior reclinate and verticals. Antennae tan, 2nd segment with several stout bristles. Arista with 3–4 dorsal branches and one below in addition to the terminal fork. Face pale yellow, carina quite low, scarcely evident. One prominent oral, the 2nd about ½ length 1st and much thinner. Cheeks yellowish, gray behind, the width about ⅓ greatest eye diameter. Palpi pale with a few short, stout hairs.

Mesonotum dark grayish brown, grayish pollinose, with a browner stripe between median acrostichal rows; scutellum and pleurae also brownish. Acrostichals in 4 rows anteriorly, in 2 rows posteriorly. Apical and basal scutellars reaching about the same distance behind. Two humerals, one strong sternopleural, the anterior one short and thin, scarcely ⅓

length of posterior one. Legs, especially femora, dark brown to black with fore coxae and all tibiae and tarsi somewhat more yellowish. Abdomen dark brown. Sternites black. Ovipositor plates dark tan with coarse, blunt teeth. Male anal plate elongate dorso-ventrally, protruding below.

Wings clear, without apical spot, the veins brown, the costa often noticeably darker. Distal costal break with 2 stout bristles; 3rd costal section with heavy spinules on its basal ½. Costal index about 3.3; 4th vein index about 1.4; 5x index about 1.2.

Length body, about 2.5 mm. (in pinned specimens); wings, about 2.8 mm.

Internal characters of imagines.

Testes of aged male bright orange, with about 1½ large outer coils and 1½ inner coils, these a bit smaller but darker. Ejaculatory sac with two quite long posterior diverticula, greatly tangled, each bifurcate at about its distal ⅓. Paragonia S-shaped.

Spermathecae with sclerotized centers, their stalks thick. Parovaria twice as large as spermathecae, elongate oval. Ventral receptacle with about 5 closely appressed loops, arranged as in *Hirtodrosophila.*

Distribution and types.—We have collected several hundred specimens in southern California, mostly from the Pasadena area, a few from Wyoming, and 18 from the mountains near Malad, Idaho. **Holotype,** ♂, from Pasadena, California, collected by the writer in sweeping in May, 1950. *Paratypes* as follows: California: Pasadena (10), Badger (1), Temecula (1); Wyoming: Kemmerer (1), Lander (2); Idaho: Malad (10).

Notes.—This species is not attracted to traps. Most of the specimens from Pasadena were taken in the writer's yard by sweeping over the lawn which, in addition to grass, contained considerable amounts of *Dichondra* and clover. Although neither larvae nor mines were found, it is believed that the clover was the host plant. We have not succeeded in raising this form in the laboratory. In addition to the localities mentioned above, Dr. Sturtevant has taken this species from Palo Alto and Truckee, Calif.

It is very difficult to describe the male genitalia. The clasper is quite large and its tooth-bearing margin is very wavy, nearly convoluted, with dense teeth, and with a dense cluster of long bristle-like teeth over its apical surface.

Scaptomyza bipunctipennis, sp. nov.

External characters of imagines.

♂. Arista with about four dorsal branches and usually one below, basal to the terminal fork. Front yellowish tan, brownish on ocellar triangle and usually on posterior orbits behind posterior orbital. Antennae, face, cheeks, palpi and proboscis pale whitish yellow, their bristles black. Carina rather low. One strong vibrissa, 2nd oral thin, usually about ½ length 1st; palpi with 2–4 stout bristles at tip. Orbitals all thin, proclinate about equal in length to posterior reclinate, anterior reclinate about ⅓ their length, its base about ⅓ nearer proclinate than posterior reclinate. Verticals,

post-verticals and ocellars long but thin. Cheeks broad, about 1/5 greatest eye diameter.

Acrostichal hairs in 4 rows anteriorly, 2-rowed between dorsocentrals with one or more hairs in dorsocentral rows enlarged. Presutural long; two humerals, the upper one longer; one strong sternopleural, the anterior one thin, scarcely 1/2 length posterior one. Mesonotum tan, pollinose, on darker specimens with a darker median stripe between the two central acrostichal rows, expanding onto scutellum to cover most of the disc. Pleurae pale tannish, darker along notopleural suture and with some discoloration on posterior portion of mesopleura and on pteropleura. Halteres pale. Apical scutellars about 2/3 length basals, directed posteriorly.

Abdomen, on darker individuals, uniformly pale brown, pollinose, semishining at some angles; on lighter specimens the darkening is present only on terminal tergites. All legs pale yellowish, with the apical tarsal joints a bit darker.

Wings clear except for a dense black cloud over apex of 3rd vein (fainter on tenerals), and, usually, with a smaller, less distinct cloud over apex of 4th vein. Two strong bristles at apex of 1st vein; 3rd costal section with heavy spinules on its basal 2/5. Costal index about 3.8; 4th vein index about 1.2; 5x index about 1.2.

Male anal plate with many long bristles above and a row of shorter hairs below; genital arch bare above, with a dense row of long bristles along lower edge and a projection from the posterior margin extending inside beneath the anal plate; clasper rectangular, its distal margin straight and bearing a row of 12–16 short, black teeth and about 15 long pale marginal bristles.

Length body, 2.8–3.2 mm. (in pinned specimen); wings, about 3 mm.

♀. Similar to male but larger and paler. Abdomen, on all individuals seen, entirely pale tan with at most darker areas laterally on tergites; anal plates black, ovipositor brown, bluntly pointed. Apical cloud on 3rd vein much smaller than on males, sometimes barely visible; no cloud at apex of 4th vein. Length body, about 3.5 mm.; wing, about 3.3 mm.

Internal characters of imagines.

Testes with about 1½ bright orange coils, rather small; paragonia weakly U-shaped, large. Ejaculatory sac not seen. Spermathecae with large, densely sclerotized centers and thick stalks; parovaria small, no larger than the centers of the spermathecae. Ventral receptacle rather tightly but irregularly coiled into a tangled mass, the basal 1/4 (estimate) very thick, about six times the thickness of the apical portion. Anal plate long haired.

Other characteristics, relationship and distribution.

Eggs.—These possess four filaments, all short, the basal pair more or less bifurcate apically, in more extreme cases giving the appearance of 3 filaments on that side.

Puparia.—Stalks of anterior spiracles very short, with about six very short branches.

Distribution and types.—We have collected 22 specimens from four localities in Calif., Wash. and Ida. The two individuals from Malad, Idaho differ in several characters from the west coast specimens and are not being included in the type series. **Holotype**, ♂, No. 2179.8, from Prairie Creek Redwood State Park, south of Crescent City, California, taken by the writer in sweeping in July, 1951. *Paratypes* as follows: California: 3, with the same data as the holotype; Washington: Kalaloch (1), Bogachiel (2). In addition, we have a number of preserved specimens which are not being considered as types.

Relationship.—This is the only North American species known to us in which the apical wing cloud differs in the two sexes. It seems to be rather closely related to an undescribed species from Argentina, known to me by eight specimens from the collections of Prof Souza Lopes of the Instituto Oswaldo Cruz, and Dr. Blanchard of Buenos Aires. In this form the males have large, dense clouds over the apices of 3rd and 4th veins, often confluent, but females possess only the cloud over the apex of the 3rd vein. Malloch (1934) has described *S. dissimilis* from South America in which the wing spots are more extremely different in the two sexes, but in other respects this form does not seem to be closely related to the present one.

Notes.—All of the individuals captured by us were taken in sweeping among grass. However, we were able to raise them in the laboratory with some difficulty. Unfortunately, our stocks were lost due to a severe bacterial infection.

Scaptomyza species B.

This is apparently an undescribed species, known to us by a single male individual from Mohawk Park, Ohio (5/10/37; H. D. Stalker), in Dr. Stalker's collection. It is not obviously related to any of the species discussed in the present paper.

Scaptomyza species F.

Several individuals of this undescribed species were seen by the writer among the unsorted flies in the collection of Dr. A. L. Melander, Riverside, Calif., who had taken them at Sequoia Park, Calif., at about 5000 feet, and at Castle Rock, Wash. The abdomen is entirely yellow, the scutellum yellow laterally, the postnotum gray pollinose and noticeably contrasting with the abdomen.

SINOPHTHALMUS Coquillett

1904. Proc. Ent. Soc. Wash., 6:190.

Genotype: *S. pictus* Coq.

This genus is represented only by the type species which is fairly widespread over the west. With the capture of additional material of *Amiota (Phortica) albavictoria*, it was noted that only a single character prevents

the present genus from coinciding exactly with *Phortica:* the bare arista. In other characters, *pictus* is remarkably similar to *albavictoria,* and almost as close to *Amiota (Phortica) variegata* of Europe. Duda (1926) has remarked on the failure of the arista character to separate adequately the various genera in this portion of the family. Although I cannot agree with his conclusions, he says (*op. cit.,* p. 246) that it is questionable whether *Erima* Kertesz and *Cocoxenus* Loew should be considered self-sufficient, and thinks it best to consider them only as subgenera of *Phortica* Schiner (= *Amiota* of the present paper), since *P. variegata* with its short-haired arista fits between *Cacoxenus* and *Erima* on the one hand (with a bare arista) and the long-haired species of *Phortica* on the other hand (e.g., *rufescens*). He states further that *Cacoxenus punctatus,* with a short-pubescent arista, is intermediate between *C. indagator* and the known species of *Phortica.* An examination of our specimens of *albavictoria* shows that the number of dorsal branches of the arista may vary from one to three, adding support to the conclusion that the character of the arista may not be a valid point of generic separation. Although the present writer feels convinced that *Sinophthalmus* will ultimately have to be placed as a synonym of *Phortica,* there is no immediate advantage in doing so at the present.

Sinophthalmus pictus Coquillett.

S. pictus Coq., 1904. Proc. Ent. Soc. Wash., 6:190.

Coquillett described the species from 12 specimens from the mountains near Claremont and from Yosemite, Calif. We have since taken it at numerous localities in California, from Yosemite south, as well as from Nevada, Arizona and New Mexico. A single specimen was taken by the writer at Zacatecas, Mexico. *S. pictus,* like many species of *Amiota,* has the annoying habit of flying in and around one's eyes and ears.

Attempts to locate the breeding places of this species in California by the writer were unsuccessful; however, with an abundant supply of specimens available, several interesting observations were made. Dr. Sturtevant recalled having dissected females and finding living larvae within the vagina. He thought that perhaps the eggs had been retained too long, and hatched internally. Acting on his suggestion a number of females were brought into the laboratory and this problem investigated.

In *Drosophila,* eggs pass from the oviduct into the vaginal cavity, coming to rest with the micropyle quite near the openings of the spermathecae and ventral receptacle. In this position, several sperm enter the egg, and it is normally laid a short time thereafter. It has been observed that in some species, e.g., *Drosophila melanogaster,* if a female is forced to retain her eggs by withholding a proper food surface for normal deposition, an egg in the vaginal cavity will proceed to develop and eventually the egg will contain a fully-formed larva which will emerge from the chorion upon deposition.

However, in *S. pictus,* such retention of eggs and subsequent deposition of fully formed larvae appears to be the rule among wild individuals,

whereas females brought into the laboratory produce a distinctly different egg which must undergo the usual period of development outside the body of the mother.

Approximately three out of four females captured would, when the abdomen was gently squeezed with a forceps, extrude a fully-developed egg, the larva immediately emerging from the thin, whitish egg membrane. On standard *Drosophila* culture medium, most of these larvae would grow and pupate in about 5–6 days. An occasional female would extrude an incompletely-developed egg, also white in color, which would hatch normally within a day or two in vials. These could be reared to pupation. Dissections of these females revealed the following characteristics: spermathecae normal for the family, with sclerotized centers; parovaria on equally long stalks, with hollow centers; ventral receptacle apparently in the shape of a short, thick sac within which the lumen appeared highly coiled, although it could not be uncoiled by pulling; ovaries with relatively few eggs and egg vesicles, the left ovary (usually) smaller than the right and with about three eggs of moderate size and two smaller, undifferentiated ones visible; right ovary much the larger, and usually with about three large well-developed eggs, nearly as large as the single ones found in the vaginal cavity itself. One may suppose that under normal conditions, only one egg is matured at a time, and that it passes into the vagina and remains there, the larva developing meanwhile. When the appropriate food source is found, the larva-bearing egg is deposited, and the larva begins its outside life at once. These facts suggest that the usual medium is either bleeding trees, in which the bleeding time is quite short, or that the flies live as parasites on some other animal.

However, some females placed in vials without having the egg expelled artificially, would eventually deposit this egg, the larva crawling away from

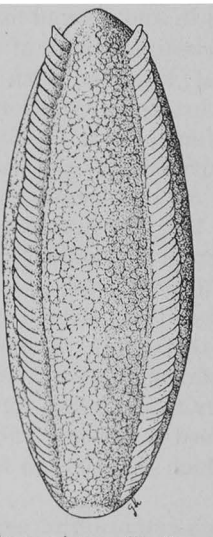


FIG. 1. Egg of *Sinophthalmus pictus*. Similar eggs are known in certain species of *Gitona* and *Rhinoleucophenga*.

it at once, and thereafter the females would lay eggs of an entirely different sort (fig. 1). These were tannish-brown in color, and possessed a pair of longitudinal "wings," fluted in appearance, on the sides of the rather flattened dorsal surface, this area being strongly marked with a raised pattern of darker brown fine ridges, roughly hexagonal in shape, though often coalescing somewhat, and not quite extending laterally to the bases of the wings. Eggs of this sort were often produced in numbers by a single female—as many as 7–11 in a short time—and were deposited on the food, on the sides of the vial, or on the cotton plugs. After a day or two these eggs would hatch, the larvae would begin to work in the food, many of them surviving to pupation. The adults resulting from such larvae were identical in every way with wild specimens and those reared from white eggs. Adults of both sexes were produced from both egg types.

Sinophthalmus pictus gagei Patterson and Mainland.

S. pictus gagei Pat. and Main., 1944. Univ. Tex. Publ. 4445:12.

This subspecies was described from a male collected by G. B. Mainland in 1942, from the Gage Ranch in central Chihuahua, Mexico. The type specimen has apparently been lost but was figured in color by the describers (*op. cit.*, Pl. XI).

STEGANA Meigen

1830. Syst. Beschr., 6:79.

Genotype: *S. coleoptrata* (Scopoli).

Our understanding of this genus is still quite unsatisfactory. There are at least three species in the U. S. but their correct names are not at all certain. Malloch and McAtee (1924) state that the two species known to them agree perfectly with two European species and assign these names to them, *viz.*, *coleoptrata* (Scopoli) and *curvipennis* (Fallén), considering *vittata* (Coquillett) a synonym of the latter. Malloch (1924b) gives a key to the New World species known to him, including several new species described briefly in synoptic form, and later (1924a) gave complete descriptions of these forms, once again reporting *curvipennis* and *coleoptrata* as American. The arrangement by the present writer seems to be the best one under the circumstances.

Stegana barretti Johnson, from Mexico, belongs to *Amiota* and is discussed under that genus.

Key to the Nearctic species of Stegana

1. Palpi largely or entirely black; 3rd antennal segment yellow or slightly discolored at tip; mesonotum yellow with more or less evident narrow black stripes; face pale yellow; apical scutellar bristles about ⅔ length basal ones ..... *vittata* (Coq.)

   Palpi entirely yellow; 3rd antennal segment black 2

2. Scutellum with white median stripe; mesonotum with about 6 brown stripes, the median pair sometimes fused ..... ? *coleoptrata* (Scop.)

   Scutellum uniformly brownish black, heavily pollinose; mesonotum dark brown, its stripes scarcely evident; legs yellow with blackish discoloration apically on femora and basally on tibiae; face yellow with dark stripe just above oral margin ..... *Sp. A*

Stegana vittata (Coquillett).

Phortica vittata Coq., 1901. Proc. U. S. Nat. Mus., 23:618.
? = *Stegana curvipennis* (Fallen).

Coquillett described *vittata* from Avalon and Delaware Water Gap, New Jersey, and from New York. A comparison of his account and specimens which agree with it with the keys and notes on European species of Duda (1924) and Seguy (1934) reveals the following striking differences:

	vittata (U.S.)	*curvipennis* (Europe)
1.	Mesonotum and scutellum yellow, former with 4–7 narrow, black lines; abdomen yellow with some brown dorsally.	Thorax and abdomen black.
2.	Legs yellowish with brown bands near apices of mid and hind femora.	Legs with 2nd femora brown.
3.	Halteres yellow.	Halteres brown.

It seems fairly certain that two entirely different flies are concerned here. Accordingly I am recognizing *vittata* as the valid name for the U. S. species.

The geographic distribution for this species cannot be given with any certainty; the records of Sturtevant (1921) are for both this species and *coleoptrata.* These records are all eastern: from Main to Florida, west to Wisconsin. Mr. Geo. Steyskal (private communication) reports *curvipennis* from Michigan.

Stegana coleoptrata (Scopoli).

Musca coleoptrata Scop., 1763. Ent. Carniol. :338.

The U. S. species going by this name is keyed on the characters given by Malloch (1924b) although, as with *vittata,* there is no assurance that this is the correct name for our species. The remarks of Duda and Seguy *(op. cit.)* on true *coleoptrata* indicate that their form may be different, possessing entirely yellow legs or with some slight darkening apically, the pleurae with a narrow brownish longitudinal stripe, the palpi brownish yellow, and the face whitish with a narrow brown band just above oral margin. According to Seguy the larvae of this small species live in wood, especially of poplar (*Populus* sp.), and the adults are attracted to the cut surfaces of oak and poplar.

The American species is known mainly from the eastern states (see above); Steyskal (private communication) reports it from Michigan, and Malloch (1921) records a female from Urbana, Ill.

Stegana species A.

Two specimens taken by the writer in general sweeping in the Dungeness Fork Forest Camp, Olympic National Forest, about 10 miles south of Sequim, Wash. (Aug., 1951), differ from the eastern species in the characters given in the key. Additional specimens from Glacier Park, Mont., and from Mt. Baker and Mt. Vernon, Wash., have been seen in the collection of Dr. A. L. Melander. We have not been able to match these with any described species but do not feel that it is wise to describe them

as new at the present time. I know of no record where members of the genus have come to banana-baited traps.

ZYGOTHRICA Wiedemann

1830. Achias Dipt., Genus 16:3.

Genotype: *Z. dispar* (Wiedemann).

The members of this genus are largely confined to Central and South America although Malloch has described a typical form from Samoa. Two species have been previously reported from Mexico and two new ones are described here. *Z. aldrichi* Stvt., known from Panama has been included in the key since it is likely that it also occurs farther north in Central America. The genus seems to be related to the subgenus *Hirtodrosophila* of *Drosophila* and, like that group, is attracted to fungi. As mentioned below, however, *Z. dispar* has been reared from flowers in Brazil.

Key to the Nearctic species of Zygothrica

1. Wings clear, without dark clouds 2
   Wings with three large clouded areas ..... *aldrichi* Stvt.
2. Head greatly extended laterally, the eyes conically produced; only two orbital bristles evident ..... *dispar* (some ♂♂)
   Head normal in shape; three orbitals present 3
3. Mesonotum brown ..... *dispar* Wied.
   Mesonotum yellowish to tan with more or less distinct darker longitudinal stripes or markings 4
4. Longitudinal stripes extending along entire length of mesonotum 5
   Mesonotum with a broad brownish stripe on posterior ⅓ which is continued across scutellum to apex; a faint stripe lateral to the large median one of mesonotum ..... *semistriata* n. sp.
5. Mesonotum with 6 longitudinal stripes, the median pair sometimes partly fused; scutellum with broad central stripe from base to apex; vibrissae arising from blackish areas ..... *poeyi* (Stvt.)
   Mesonotum with 3 broad stripes and two faint lateral ones, the middle 3 completely fusing at about the level of the anterior dorsocentrals; scutellum with a subquadrate dark area basally, the periphery including apex, pale yellowish tan; face wholly pale yellow ..... *scutellaris* n. sp.

Zygothrica aldrichi Sturtevant.

Z. aldrichii Stvt., 1920. Proc. U. S. Nat. Mus., 58:157.

Sturtevant described this species from 33 individuals taken by Busck along the Trinidad River, Panama, from a white toadstool. He also reports it from Trinidad, West Indies. It probably occurs in Mexico.

Zygothrica dispar (Wiedemann).

Achias dispar Wied., 1830. Aussereurop. Zweifl., 2:556 (Diopsidae).

This species was described from Brazil and is known from Panama, West Indies and Mexico. The strangely pointed heads of some males are quite remarkable in the family and deserve further investigation since Sturtevant (1920) points out that the head character is variable, some males having the rounded eyes and orbital bristles found in females. The species has been found in association with fungi on several occasions; how-

ever, Frota-Pessoa (private communication) states that he has reared adults from flowers of *Brunfelsia grandiflora* (Solanaceae) from the Botanical Garden in Rio de Janeiro, Brazil. He has kindly donated several of these specimens to our collection.

Zygothrica poeyi (Sturtevant).

Drosophila poeyi Stvt., 1921. Carnegie Inst. Wash. Pub. 301:76.

Originally described from Havana, Cuba, the collectors from this laboratory have taken the species in the following states in Mexico: Mexico, Michoacan, Puebla, and Federal District. Most of these collections were made from fungi.

Zygothrica scutellaris, sp. nov.

External characters of imagines.

♂. Arista with 3–4 dorsal branches and 1 branch below basal to the terminal fork. Front longer than wide, the orbits pale yellowish tan with a large semi-shining, light brown frontal triangle reaching to the lunule, bordered on either side by rather wide, blackish-brown stripes, paler anteriorly. Verticals, postverticals and ocellars long, orbitals thin, anterior reclinate about ⅔ length posterior reclinate and slightly nearer the latter than the proclinate one, all 3 in nearly a straight row. Antennae light tan, 3rd joint darker, rather large and thickly haired, 2nd segment with 1 stout dorsal bristle and several smaller ones. Face and cheeks pale tannish yellow; carina large, prominent, rounded; antennal grooves beside carina deep. Palpi and proboscis yellow. One strong vibrissa, other orals short. Cheeks about ¼ greatest eye diameter; eyes red with short, inconspicuous, pale pile.

Acrostichals in about 8 irregular rows; no prescutellars. Anterior dorsocentrals close to posterior ones, scarcely ⅔ as long. Anterior scutellars convergent, nearly as long as apical pair. Two humerals, ventral one short; 2 sternopleurals, anterior one about ⅓ length posterior. Mesonotum light tannish yellow with dark brown longitudinal stripes as follows: one covering median 4 acrostichal rows, and one in each dorsocentral row, these three coalescing on posterior ½–⅓ of mesonotum, this large brown area continuing onto scutellum forming a rectangular basal area on about half of the disc, the remainder of the scutellum pale yellow; there are less distinct stripes above the alar bristles and also anteriorly along upper side of humeri. Pleurae pale yellow, or with slight discoloration just below notopleural suture. Halteres and legs yellow; fore tarsi with many short, irregular, recurved hairs.

Abdomen yellow with black bands; 1st 2 tergites nearly all yellow, the next 2 with large, black, shining bands, covering the tergite centrally but narrowing laterally into pointed apical extensions of the bands which fail to reach the margin; the following tergite with a large, black, shining area only in middle, the next showing a still smaller median spot, while the genital arch is entirely black except on the lower apical corners.

Wings clear, unmarked. Bristles at distal costal break thin and fine, the dorsal one larger; 3rd costal section with heavy bristles along its basal ⅗ or a bit less; costal index about 2.5; 4th vein index about 1.5; 5x index about 0.6, the last section of the 5th vein being quite long.

Length body (in pinned specimens) : about 2.5 mm.

Length wing: 2.8 mm.

Distribution and *Types.*—**Holotype** male (No. 1344.10) from Laguna Patzcuaro, Mich., Mexico, collected by G. B. Mainland, 8-1-42. *Paratype* male (No. 1342.9) collected by G. B. Mainland at Desietro de los Leones, D. F., Mexico, 7-29-42.

Zygothrica semistriata, sp. nov.

External characters of imagines.

♂. Arista with 4–5 dorsal branches, and 1 branch below basal to the terminal fork. Front light tannish-brown, lighter in midline and pale anteriorly, yellowish pollinose on orbits, blackened on ocellar triangle; no enlarged prominent frontal triangle. Antennae tannish, moderately long-haired, 3rd joint long, reaching about to oral margin. Carina very large, especially prominent below, rounded, the face on either side deeply sunken forming crypts in which the antennae lie. Carina tan above, distinctly blackened below and along the incised oral margin. One strong vibrissa arising at the lower edge of the antennal foveae. Cheeks broad below center of eye, nearly ½ greatest eye diameter; cheeks dirty yellow, a tannish area just below center of eye, the lower margin black. Palpi dark, with a long apical bristle. Anterior reclinate orbital thin, about ⅓ length proclinate and closer to the latter than to posterior reclinate.

Acrostichal hairs in 6 rows, in 4 rows between the dorsocentrals; no prescutellars. Anterior dorsocentrals ⅔ length posterior pair; the 4 scutellar bristles about equal in length. Two strong humerals, the upper one longer; 2 sternopleurals, the anterior a little more than half length posterior; no propleurals. Disc of mesonotum tan, densely pale pollinose, becoming dark brown between dorsocentrals and similarly darkened over most of scutellum, only the lateral bristle-bearing areas pale yellow; less distinct darker stripes between dorsocentrals and supra-alars. Pleurae largely tan, indistinctly brownish on sternopleurae. Halteres pale. Legs uniformly pale yellowish-tan. Fore tarsi with pairs of semi-erect hairs along outer surface as in many species of *Scaptomyza;* fore metatarsus with several longer hairs below. Distinct apicals on 2nd tibiae, preapicals on 3rd tibiae, 1st tibiae without either obviously present.

Abdomen yellow with black bands which cannot be described accurately from these pinned specimens. The bands appear to decrease in size on posterior segments but retain median extensions to the base of the tergites; the lateral areas are also darkened except on the pregenital tergite. Male anal plate rather sparsely long-haired, with an elongate projection below as figured for *Z. poeyi* (Hsu, 1949).

Wings with a uniform brownish tinge, veins dark. Distal costal break with 2 bristles, the dorsal one distinctly stouter. Third costal section with

heavy bristles along its basal ½ or slightly less. Costal index about 2.8; 4th vein index about 1.7; 5x index about 2.2.

Length body (in pinned specimens) : about 3 mm.

Length wing: 3.5 mm.

Distribution and Types.—Known from two males collected by the writer from fungus at Peña de Gato, Puebla, Mexico in Sept., 1947. From general sweeping in the same area 5 specimens of *Z. poeyi* were captured.

Holotype male and *paratype* male (No. 1800.7), from the above locality, in the collection of The University of Texas.

GENUS X

This genus, apparently undescribed, is known to me by two specimens as follows: ♂, Kern Canyon, Calif., April, 1934 (Th. Dobzhansky), now in the collection of Dr. A. H. Sturtevant; ♀, mountains about 25 miles northwest of Las Vegas, Nev., June, 1948, collected by the writer. These specimens seem to represent the same species and are rather intermediate between *Leucophenga* and *Rhinoleucophenga*, simulating the latter more noticeably in size, color and general body build. The following brief description may facilitate its future recognition:

Large yellowish species; arista plumose with short ventral branches. All orbitals strong; postverticals large; vibrissae large followed by a row of long, stout bristles. Carina quite low, nearly absent; cheeks narrow. Anterior dorsocentrals short; strong prescutellars; 1 strong humeral; 2 sternopleurals. Wings large, costa reaching 4th vein but weak beyond 3rd; wing blade rather dark with clouds over both crossveins and weaker ones along distal half of 1st vein and at apices of 2nd and 3rd veins. Abdomen pale brownish to tan, without obvious banding. Body length, ♂, about 4.5 mm. in pinned specimen.

REFERENCES

Brues, C. T., and A. L. Melander. 1945. Classification of Insects. Cambridge, Mass., Harvard Univ. Press. 672 pp.

Collin, J. E., 1911. Additions and corrections to the British list of Muscidae Acalyptratae. Ent. Mon. Mag., ser. 2, 22:229–234.

Coquillett, D. W., 1900. Report on a collection of Dipterous Insects from Puerto Rico. Proc. U. S. Nat. Mus., 22:249–270.

Costa Lima, A. 1935. Um Drosophilideo predador de Coccideos. Chac. e Quint., 52:61–63.

Curran, C. H., 1928. Scientific survey of Porto Rico and the Virgin Islands. Vol. 11, pt. 1. Insects of Porto Rico and the Virgin Islands. Diptera or two-winged flies. New York, N. Y. Acad. Sci. 118 pp.

Curran, C. H., 1934. The Diptera of Kartabo, Bartica District, British Guiana. Bull. Amer. Mus. Nat. Hist., 66:287–523.

Doering, K. C., 1942. Host plant records of Cercopidae in North America, north of Mexico (Homoptera). Jour. Kans. Ent. Soc., 15:65–92.

Duda, O., 1924. Beitrag zur Systematik der Drosophiliden unter Berücksichtigung der paläarktischen u. orientalischen Arten (Dipteren). Arch. f. Naturg., 90 A 3:172–259; 7 Pl.

Duda. O., 1925. Die costarichanischen Drosophiliden (Diptera) des ungarischen National-Museums Zu Budapest. Ann. hist.-nat. Mus. hung., 22:149–229.

Duda, O., 1926. Die orientalischen und australischen Drosophiliden-Arten (Dipteren) des ungarischen National-Museums du Budapest. Ann. hist.-nat. Mus. hung., 23:241–250.

Duda, O., 1927. Die südamerikanischen Drosophiliden (Dipteren) unter Berücksichtigung auch der anderen neotropischen sowie nearktischen Arten. Arch. f. Naturg., 1925, 91 A 11–12:1–228.

Frost, S. W., 1924. A study of the leaf-mining Diptera of North America. Cornell Univ. Agr. Exp. Sta. Memoir 78:1–228.

Frota-Pessoa, O., 1947. Revisão do gênero *Clastopteromyia*. Sum. Bras. Biol., 1:181–241.

Frota-Pessoa, O., and M. R. Wheeler, 1951. A revision of the genus *Neotanygastrella* Duda (Diptera, Drosophilidae). Rev. Bras. Biol., 11:145–151.

Hendel, F. H., 1928. Über die minierenden europäischen Scaptomyza-Arten und ihre Biologie (Diptera). Zool. Anzeig., 76:289–302.

Hendel, F. H., 1933. Neue acalyptrate Musciden aus der paläarktischen Region (Dipt.). Deutsch. ent. Zeit., 1933:39–56.

Hsu, T. C., 1949. The external genital apparatus of male Drosophilidae in relation to systematics. Univ. Tex. Publ. 4920:80–142.

Judd, W. W., 1949. Insects collected in the Dundas Marsh, Hamilton, Ontario, 1947–48. Jour. N. Y. Ent. Soc., 57:225–231.

Kröber, O., 1912. Beitrag zur Biologie der Drosophilinae. Zeit. wissen. Insektenbiol., 8:235–236; 329.

Malloch, J. R., 1915. Some additional records of Chironomidae for Illinois and notes on other Illinois Diptera. Bull. Ill. State Lab. Nat. Hist., 11:305–363; 5 Pl.

Malloch, J. R., 1921. Some notes on Drosophilidae (Diptera). Ent. News, 32:311–312.

Malloch, J. R., 1924a. Descriptions of neotropical two-winged flies of the family Drosophilidae. Proc. U. S. Nat. Mus., 66:1–11.

Malloch, J. R., 1924b. The American species of the Drosophilid genus *Stegana*. Ent. News, 35:96–100.

Malloch, J. R., 1926. New genera and species of acalyptrate flies in the United States National Museum. Proc. U. S. Nat. Mus., 68:1–35.

Malloch, J. R., 1932. New species and other records of Otitidae (Ortalidae), Piophilidae, Clusiidae, Chloropidae and Drosophilidae from the Marquesas. (Marquesan Insects, I). Bull. Bishop Mus., 98:205–223.

Malloch, Jr R., 1934a. Diptera of Patagonia and South Chile. Pt. VI, Fasc. 5, London, Brit. Mus. (Nat. Hist.). 489 pp.

Malloch, J. R., 1934b. Insects of Samoa. Pt. VI. Diptera, Fasc. 8. Drosophilidae, Ephydridae, Sphaeroceridae and Milichiidae. London, Brit. Mus. (Nat. Hist.). pp. 267–328.

Malloch, J. R., and W. L. McAtee, 1924. Flies of the family Drosophilidae of the District of Columbia region, with keys to genera, and other notes, of broader application. Proc. Biol. Soc. Wash., 37:25–42.

Malogolowkin, C., 1946. Sôbre o gênero "Rhinoleucophenga" com descrição de cinco espécies novas. Rev. Bras. Biol., 6:415–426.

Melander, A. L., 1913. A synopsis of the dipterous groups Agromyzinae, Milichiinae, Ochthiphilinae and Geomyzinae. Jour. N. Y. Ent. Soc., 21:219–273; 283–300.

Patterson, J. T., 1943. The Drosophilidae of the Southwest. Univ. Tex. Publ. 4313:7–214.

Patterson, J. T., and G. B. Mainland, 1944. The Drosophilidae of Mexico. Univ. Tex. Publ. 4445:9–101.

Quayle, H. J., 1938. Insects of Citrus and other Subtropical Fruits. Ithaca, N. Y. Comstock Pub. Co. 583 pp.

Sabrosky, C. W., 1951. Two new species of *Pseudiastata* (Dipt., Drosophilidae) predacious on the pineapple mealybug. Bull. Ent. Res., 41:623–627.

Seguy, E., 1934. Dipteres (Brachyceres). Muscidae Acalypterae et Scatophagidae. *in* Fauna de France, Vol. 28. Paris, Lechevalire et Fils. 832 pp.; 27 Pl.

Seguy, E., 1951. Ordre des Dipteres. *in* Traite de Zoologie. Insectes Superieurs et Hemipteroides. Tome X, Fasc., 1:449–744.

Stalker, H. D., 1945. On the biology and genetics of *Scaptomyza graminum* Fallén (Diptera, Drosophilidae). Genetics, 30:266–279.

Steyskal, G. C., 1949. A new anomalous acalyptrate fly (Diptera). Bull. Brookl. Ent. Soc., 44:134–137.

Sturtevant, A. H., 1918. A synopsis of the Nearctic species of the genus *Drosophila (sensu lato)*. Bull. Am. Mus. Nat. Hist., 38:441–446.

Sturtevant, A. H., 1920. The dipterous genus *Zygothrica* of Wiedemann. Proc. U. S. Nat. Mus., 58:155–158.

Sturtevant, A. H., 1921. The North American species of *Drosophila*. Carneg. Inst. Wash. Publ. 301. 150 pp.

Sturtevant, A. H., 1942. The classification of the genus *Drosophila*, with descriptions of nine new species. Univ. Tex. Publ. 4213:5–51.

Wheeler, M. R., 1947. The insemination reaction in intraspecific matings of *Drosophila*. Univ. Tex. Publ. 4720:78–115.

Wheeler, M. R., 1951. *Dettopsomyia* and *Ptilomyia:* two genera new to the United States. Pan-Pac. Ent., 27:92–94.

XII. THE EFFECT OF TWO PERICENTRIC INVERSIONS UPON CROSSINGOVER IN *DROSOPHILA MELANOGASTER*

MARY L. ALEXANDER

INTRODUCTION

Chromosome variation within the Genus Drosophila due to inversions across the centromere, pericentric inversions, is rather extensive. An analysis of five subgenera and unclassified groups by Wharton (1942, 1943) showed that the number and length of the euchromatic arms of the salivary gland chromosomes and metaphase configurations were modified in some fifteen species and two subspecies by such inversions. Several other cases have been reported by Kikkawa (1936), Burla et al. (1949) and Ward (1949). Although in most of these cases the autosomes were involved, Wharton (1943) reports four cases in which the sex chromosome has been changed to a V or J shape. Examples of chromosome evolution by the occurrence of pericentric inversion have thus been found in the four large subgenera of the Genus Drosophila. These are the subgenera Hirtodrosophila Duda, Pholadoris Sturtevant, Sophophora Sturtevant and Drosophila Fallén. The general problem of chromosome evolution in Drosophila has been discussed by Patterson and Stone (1952).

The presence of heterozygous pericentric inversion in natural populations of *Drosophila algonquin* (Miller, 1939) and *Drosophila robusta* (Carson and Stalker, 1947) strengthens the suggestion that changes in the centromere position on the chromosome have been produced by pericentric inversions. These cases prove that some types of mechanisms allow the retension of this type of inversion even heterozygous in populations.

The present investigation tests the affect of two X-ray induced pericentric inversions in the second chromosome of *Drosophila melanogaster* on crossing over, disjunction and the production of aneuploid gametes. One, *Glazed,* involves almost equal segments on each side of the centromere, while the *Plum*^{2} inversion extends from a break very close to the centromere in 2L to a point near the free end of 2R. These represent two general types of pericentric inversions encountered in studies of chromosome variation.

MATERIAL AND METHODS

The cytological position of the mutant markers, which were used, and of the points of breakage of the *Glazed* and *Plum*^{2} inversions are presented in Figure 1. The approximate cytological positions on the salivary gland chromosome maps of Bridges (1935) for the mutants of the test stock, *aristaless, dumpy, black, purple, curved, plexus, speck,* are known except for *curved.* The crossover map position is indicated above each mutant.

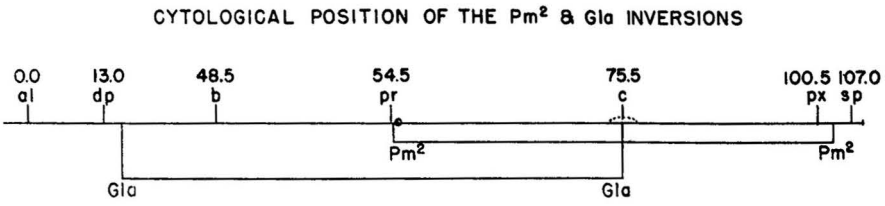


FIGURE I

The *Glazed* inversion involves about 60 percent of the physical length of the salivary gland chromosome. The breaks occur in the euchromatic portion of 2L and 2R and almost equal segments of both arms are affected. The breaks were determined as 27E and 51D by Morgan, Bridges and Schultz (1936). The left break lies between the loci of *dumpy* and *black;* the exact position of the locus of *curved* in relation to the inversion has not been determined cytologically but it is close to the break in 2R. The *Plum*^{2} inversion principally involves the 2R arm. The left break occurs at 40F very close to the centromere and induces a position effect with *light,* and the right break lies between *plexus* and *speck* in the 59E region approximately at the *brown* locus (Schultz and Dobzhansky, 1934). About fifty percent of the salivary gland chromosome is included in this inversion.

To test the effect of these inversions upon crossingover, females heterozygous for one of them and *al dp b pr c px sp* were crossed in pairs with *Cy/al dp b pr c px sp* males. Crossingover was determined in the non *Curly* progeny. The occurrence of aneuploidy resulting from single and certain multiple crossovers within the limits of the inversion was detected by egg counts. The inversion stocks and three wild strains of *melanogaster,* Stephenville, Oregon R and UT 1930 (homozygous normal), were utilized in the following way. Heterozygous males and females were produced by mating Oregon R females to Stephenville males and the inversion stocks to the UT 1930 strain. Control and test crosses were made by mating males (control) and females (test) which were heterozygous for the inversion to the Oregon R/UT 1930 stock. Egg counts were made by use of some 16 to 30 pairs for each of the four tests. A minimum number of 100 eggs and maximum of 300 was counted from each pair.

RESULTS

The crossover data are presented in Table 1. The *Glazed* inversion which showed complete suppression of double crossingover gave the following results in egg counts: controls, 3770/4050 = 93.1 percent hatch; test crosses, 4299/4975 = 86.4 percent hatch, a reduction in hatch of 6.7 percent. The crossingover suppression is not limited to the inverted region since no exchanges were detected for regions one and two (Table 1). Only about 11% and 27.5% of the expected amount of crossingover

occurred in regions five and six, respectively. About 88.9% reduction for region five and 72.5% for region six thus occurred (Table 2).

TABLE 1

Crossover Counts from Heterozygous Inversions

Inversion ♀ / *al dp b pr c px sp*	*Cy (pr)* / *al dp b pr c px sp* ♂	
	Glazed	*Plum 2*
Non crossovers		
Inversion	1271	840
al dp b pr c px sp	757	440
Single—Region 1		
dp b pr c px sp	0	122
al Inversion	0	174
Single—Region 2		
b pr c px sp	0	409
al dp Inversion	0	346
Single—Region 3		
pr c px sp	0	90
al dp b Inversion	0	93
Single—Region 4		
c px sp	0	0
al dp b pr (Inversion?)	0	0
Single—Region 5		
px sp Inversion	29	0
al dp b pr c	30	0
Single—Region 6		
sp Inversion	21	0
al dp b pr c px	17	0
Doubles 1–2		
al b pr c px sp	0	5
dp Inversion	0	7
Doubles 1–3		
al pr c px sp	0	6
dp b Inversion	0	16
Doubles 2–3		
al dp pr c px sp	0	10
b Inversion	0	19
Doubles 2–6		
b pr c px	0	0
al dp sp Inversion	0	1
Doubles 4–5		
al dp b pr px sp	0	4
c Inversion	0	4
Doubles 4–6		
al dp b pr sp	0	0
c px Inversion	0	1
Triples 1–4–5		
dp b pr px sp	0	1
al c Inversion	0	1
Triples 1–5–6		
dp b pr c sp	0	1
al px Inversion	0	0
Triples 2–4–5		
b pr px sp	0	6
al dp c Inversion	0	5
Triples 3–4–5		
pr px sp	0	1
al dp b c Inversion	0	1
Quadruple 2–3–4–5		
al dp pr px sp	0	0
b c Inversion	0	2
Total	2125	2605

The *Plum*^{2} inversion allowed an appreciable amount of double crossingover within the limits of the inversion. Table 1 shows that 27 individuals which resulted from such crossingover were recovered from the total of 2,605 tested. Cases in which one break obviously occurred outside the limits of the inversion as Double 2–6 were disregarded for the determination of the total amount of double crossingover within the inversion. The difference in 94.7 percent hatch (4651/4913) for the control crosses and 84.8 percent (4774/5628) for the test crosses must represent the aneuploid gametes with the strands produced by single and some multiple exchanges within the inversion. When the value of 2.1 percent for single crossover strands resulting from double exchange is removed, the difference of 9.9 percent in hatch is reduced to 7.8 percent. The *Plum*^{2} inversion produces some slight reduction in crossover in region 1 (1.7 percent reduction) and 2 (12.5 percent reduction). The third region, that between *black* and *purple*, which is adjacent to the left break shows an increase of crossingover. The remaining regions, four, five and six, which are included within the inversion show a reduction in crossingover of from 95 to 98 percent. The latter region is only partially included since it contains the right break of the inversion. All the data on the reduction in double crossingover within the inversion and single crossingover along the remaining portion of the chromosome for the *Plum*^{2} inversion agree with that obtained by Schultz and Dobzhansky (1934).

TABLE 2

Reduction in Crossingover Percent by the *Glazed* and *Plum-2* Inversions

	Region 1 *al-dp*	Region 2 *dp-b*	Region 3 *b-pr*	Region 4 *pr-c*	Region 5 *c-px*	Region 6 *px-sp*
CROSSOVER UNITS						
Standard	13.0	35.5	6.0	21.0	25.0	6.5
Glazed	0.0	0.0	0.0	0.0	2.77	1.78
Plum-2	12.78	31.05	9.14	0.99	0.99	0.11
REDUCTION IN CROSSOVERS (Percent)						
Glazed	100.00	100.0	100.0	100.0	88.9	72.5
Plum-2	1.7	12.54	-52.3	95.3	96.0	98.2

DISCUSSION

Chromosome evolution can occur by means of several different mechanisms. The fusion of two rods or a rod and a dot to form a V or J which reduces the number of chromosome pairs seems to be fairly frequent in the Genus Drosophila (Patterson and Stone, 1952). There are numerous examples of such reduction in the number of chromosome pairs, but the only case of an increase in centromere number in wild populations is that in *Drosophila trispina* reported by Ward (1949). Here an increase in the centromere number has been realized such as that produced experi-

mentally in *D. melanogaster* by Stone and Griffen (1940) although the method of attainment of this condition in *trispina* can only be inferred.

Other than a change in the number of centromeres, the metaphase and salivary morphology may be changed by the acquisition or loss of heterochromatin, variation in the Y chromosome and an increase in the number of euchromatic arms by pericentric inversions (Wharton, 1943). The survival of pericentrics as reflected in the chromosome evolution of this Genus clearly indicates that this type of inversion has not been a rare occurrence or restricted to any one species group. Of the seventeen cases of pericentric produced V-shaped chromosomes reported by Wharton (1943) nearly half, seven, of the species have acquired seven euchromatic arms in the salivary gland chromosome indicating that pericentrics had occurred in two different chromosomes. In another case, *Drosophila duncani,* three pericentric inversions, one in the X and two in the autosomes, resulted in eight euchromatic arms in the salivary chromosomes. Some of these cases could be explained by the less probable explanation of two independent translocations involving the same two chromosomes, or by centromere shifts.

Not only can there be attained a redistribution of genetic material within the same chromosome element by pericentrics but also an exchange between two elements if these are carried on the same centromere. Wharton (1942, 1943) relates such a mechanism, or a translocation, to the presence of extra long rods in *Drosophila spinofemora, Drosophila testacea* and *Drosophila tranquilla.* In the willistoni group two different combinations of the basal and terminal segments of the two arms of the second chromosome were found by Burla et al. (1949). An inversion across the centromere has taken place in the phylogny of these sibling species to recombine the basal and terminal portions of 2L and 2R. *Drosophila willistoni* and *Drosophila tropicalis* were found to have similar combinations of terminal and basal segments which differed from two other species, *Drosophila paulistorum* and *Drosophila equinoxialis,* both of which showed the second combination. This case clearly supports the suggestion by Wharton that segments of two different elements could be intermingled by pericentric inversions.

The main objection to pericentric inversions as an explanation for the change of the position of the centromere on the chromosome is the production of duplication-deficiency chromatids as a result of single exchange within the limits of the inversion. The pericentric inversions have no known meiotic mechanism which eliminates the aneuploid chromatids such as that which exists in the case of paracentric inversions (Sturtevant and Beadle, 1936). This difficulty can be reduced by the assumption that instead of two breaks, as is required for a pericentric inversion, three breaks occur and the segments of the chromosome reunite in such a way that the centromere occupies a new position on the chromosome. In Drosophila there is no crossingover in the males, nor does the presence of heterozygous inversions cause an appreciable amount of non-disjunc-

tion. In the present experiments the reduction in viable offspring of six to seven percent due to all causes in the control where the male inversion heterozygotes were tested does not indicate an appreciable amount of induced non-disjunction by the inversions. In female Drosophila localization of chiasmata does occur for crossingover is much reduced in the region of the centromere. The centromere seems to be responsible for this reduction.

The first pericentric in wild populations was found in *Drosophila algonquin* by Miller (1939). As Miller suggests the association of this arrangement with two overlapping paracentric inversions probably reduces crossingover so that few aneuploid chromatids are formed. Crossingover within reinverted portion produced by the overlapping inversions results in bridge-fragment chromatids which are eliminated harmlessly as in the case of paracentric inversion in Drosophila (Carson and Stalker, 1947).

The two pericentric inversions recovered by Carson and Stalker (1947) in wild populations of *Drosophila robusta* involved rather long segments of the second and third chromosome. These are not always associated with overlapping paracentric inversions. The 3R–L pericentric involves breaks in the two euchromatic arms of the third chromosome and is of the same general type as the *Glazed* inversion in *melanogaster*. It has been recovered from only one locality, Big Fish Lake, Minnesota.

The 2L-R inversion is the same type of pericentric as *Plum*^{2}—that is one break is very close to the centromere and the other well out in the euchromatic arm. This inversion shows a high frequency in the northern part of the United States with a general decrease toward its southern most limit at Gatlinburg, Tennessee. Carson and Stalker (1947) reported a frequency of 30.8% in the Mt. Vernon, Iowa, population and Levitan (1951) found a frequency of 24.8 to 38.6 percent in the Englewood Cliffs, New Jersey, population. The Big Fish Lake, Minnesota, sample proved to be homozygous for the 2L-R inversion thus changing the metaphase configuration of the second chromosome from a middle-sized V to a J (Carson and Stalker, 1947). Apparently the persistence of this pericentric requires a different explanation than does that of the one in *algonquin*. However, at the present time, the lack of any data on the frequency of aneuploid chromatids produced and the absence of any apparent crossover suppressor mechanism as overlapping inversions leave any tentative explanation still in question.

The reduction in crossingover observed in the *Plum*^{3} and *Glazed* inversions can not be explained by Dobzhansky's "attraction" hypothesis of homologous loci (Dobzhansky, 1931). In these and in other long inversions the pairing of homologous loci within the inverted segment should not be affected enough to lead to such an extreme reduction in crossingover. Although this hypothesis does not explain the reduction in crossingover within long inversions, there exists the possibility of an interference in the pairing of the inverted segments of short inversions. As suggested by Sturtevant and Beadle (1936) : "A short inversion may be supposed to have its pairing more interfered with by the uninverted sections than does a long inversion which has shorter uninverted sections." Less crossingover

would then be expected in short inversions because of unpaired regions than in the long inversions such as *Glazed* and *Plum*^{2} and the aneuploidy produced from single crossovers should be reduced. Despite the fact that *Glazed* and *Plum*^{2} are long inversions, the egg hatch reduction due to the production of aneuploid gametes was less than ten percent in each case.

The success of pericentric inversions in natural populations obviously depends primarily on the amount of crossingover which occurs unless some now unknown meiotic mechanism exists. There was no significant discrepancies in the two classes recovered for crossover regions in *Plum*^{2}. This minimizes the possibility of non-random disjunction at the second meiotic division such as that found by Novitski (1951) for ring and attached X chromosomes and dissimilar rods.

The *Glazed* and *Plum*^{2} inversions reduce crossingover to a different degree. Although *Glazed* is a slightly longer inversion than *Plum*^{2}, less crossingover occurs in the heterozygous condition. The position of the *Glazed* inversion on the chromosome possibly determines this difference, but the differences found for paracentrics with respect to crossingover (Stone and Thomas, 1935; Sturtevant and Beadle, 1936) demands additional tests of pericentrics to support this point. Even though these two pericentrics do vary in the reduction of crossingover both produce a comparatively small amount of aneuploidy. No general statement can be made on the amount of aneuploidy expected from heterozygous pericentrics in natural populations, but the low amount from these long inversions suggests that it may be a much smaller effect than had been supposed. Until other three break rearrangements are demonstrated, the high effective fertility of these very long pericentrics makes unnecessary an assumption that three break centromere shifts are a factor in chromosome evolution in Drosophila.

REFERENCES

Bridges, C. B. (1935). Salivary Chromosome Maps. With a Key to the Banding of the Chromosomes of *Drosophila melanogaster*. Journal of Heredity 26: 60–64.

Burla, H., Brito da Cunha, A., Cordeiro, A. R., Dobzhansky, Th., Malogolowkin, C. and Pavan, C. (1949). The *willistoni* Group of Sibling Species of Drosophila. Evolution Vol. III; 300–314.

Carson, H. L. and Stalker, H. D. (1947). Gene Arrangements in Natural Populations of *Drosophila robusta* Sturtevant. Evolution Vol. 1, No. 3; 113–133.

Dobzhansky, Th. (1931). The Decrease of Crossing-over Observed in Translocations, and its Probable Explanation. American Naturalist, Vol. LXV; 214–232.

Kikkawa, H. (1936). Two races of *Drosophila montium*. Jap. Jour. Genetics, 12; 137–142.

Levitan, Max (1951). Experiments on Chromosomal Variability in *Drosophila robusta*. Genetics 36; 285–305.

Miller, D. D. (1939). Structure and Variation of the Chromosomes in *Drosophila algonquin*. Genetics 24; 699–708.

Morgan, T. H., Bridges, C. B., Schultz, J. (1936). Report of Investigations on the Constitution of the Germinal Material in Relation to Heredity. Carnegie Year Book 35: 289–297.

Novitski, E. 1951. Non-random disjunction in Drosophila. Genetics, 36:267–280.

Patterson, J. T. and Stone, W. S. (1952). Evolution in the Genus Drosophila (in press).

Schultz, J. and Dobzhansky, Th. (1934). The Relation of a Dominant Eye Color in *Drosophila melanogaster* to the Associated Chromosome Rearrangement . Genetics 19: 344–364.

Stone, W. and Thomas, I. (1935). Crossover and Disjuntional Properties of X Chromosome Inversions in *Drosophila melanogaster*. Genetica XVII; 170–184.

Stone, W. S. and Griffen, A. B. (1940). Changing the Structure of the Genome in *Drosophila melanogaster*. The University of Texas Publication 4032: 208–217.

Sturtevant, A. H. and Beadle, G. W. (1936). The Relations of Inversions in the X Chromosome of *Drosophila melanogaster* to Crossing Over and Disjunction. Genetics 21: 554–604.

Ward, C. L. (1949). Karyotype Variation in Drosophila. The University of Texas Publication 4920: 70–79.

Wharton, Linda T. (1942). Analysis of the Repleta Group of Drosophila. The University of Texas Publication 4228: 23–52.

Wharton, Linda T. (1943). Analysis of the Metaphase and Salivary Chromosome Morphology Within the Genus Drosophila. The University of Texas Publication 4313: 282–303.

XIII. PHENOTYPIC ABNORMALITIES OF THE EYES OF *LOZENGE* ALLELES IN *DROSOPHILA MELANOGASTER*

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INTRODUCTION

A series of alleles provides excellent material for an approach to the problem of genic action. In many cases several apparently unrelated adult structures are altered as the result of the pleiotropic effects of a single gene. A study of the development of the mutant often indicates an early structural alteration which leads to these adult abnormalities. Analysis of the pleiotropic effects in a group of multiple alleles contributes valuable results to this problem since such a study makes available data regarding genes which produce the same effects in varying degrees.

The lozenge complex is composed of a series of X-linked pleiotropic alleles in *Drosophila melanogaster* which are excellent for such a study. The most striking effects produced by each allele are variation in the shape and size of the compound eyes and the abnormal distribution of eye pigment (Gottschewski, 1936; Oliver, 1947), but the mutants may also decrease the fertility of the females (Oliver and Green, 1944; Anderson, 1945) and cause structural abnormalities of the tarsal claws and pulvilli (Cummings, 1946).

Several comparative studies have been made on the lozenge series. Anderson (1945) studied the development of the female reproductive tract in the lozenge alleles to analyze the cause of decreased fertility. He reported that the decreased fertility was associated with the inviability of the sperm, and his developmental studies revealed that the sperm inviability within the females was correlated with the degree of structural abnormality of the spermathecae and parovaria. Some of the lozenge alleles cause complete absence of the spermathecae and parovaria in the females, but other alleles condition development resulting in abnormal shape and size of the structures. The fertility factor, therefore, is only indirectly an effect of the gene, the degree of expression being dependent upon the extent of structural abnormality produced in the female reproductive tract. Oliver (1947) described the variations and seriation in the surface structure and pigment distribution of ten lozenge alleles, and Green (1948) made a quantitative analysis of the amount of red pigment in the eyes of a number of the lozenge mutants.

No histological study has been made on this series from a comparative viewpoint. Chen (1929) measured the optic discs in the larvae and early pupae of wild-type *Drosophila* and *lozenge-III*; Waddington and Pilkington (1943) studied the adult and pupal stages of four mutants, *facet, morula, lozenge-spectacle,* and *opthalmopedia.* Although comparisons were made by these workers between the structure of the normal eye and a

single lozenge mutant, no attempt was made to contrast these mutants with any of their alleles.

It is the purpose of this problem to determine the type and the extent of structural abnormalities of the eyes produced by each of the lozenge alleles, and to determine the degree of correlation between the histological abnormalities and the phenotypic expressions of the alleles in the adult flies.

MATERIALS AND METHODS

The Stephenville strain of *Drosophila melanogaster* was used for the study of the wild-type compound eyes. The mutant stocks used were: *lozenge (lz)*, the original mutant at locus 27.7, *lozenge-III* (lz^3), *lozenge-34* (lz^{34}), *lozenge-36c* (lz^{36c}), *lozenge-37* (lz^{37}), *lozenge-glossy* (lz^g), and *lozenge-spectacle* (lz^s), all described by Bridges and Brehme (1944), and *lozenge Bar-Stone* (lz^{BS}), *lozenge spectacle-Bishop* (lz^{sB}), and *lozenge-y4* (lz^{y4}), which were described by Oliver (1947). All lozenge stocks were balanced with the *ClB* inversion; lz^{y4} was also carried in a balanced form by using an *attached-X* stock bearing *yellow, vermilion,* and *forked.* All flies used in the study were raised on the standard banana-yeast-malt-agar laboratory food and maintained at a temperature of approximately 23° C.

For the histological analysis of the fully developed eyes, heads of etherized adult males were removed and fixed for 30 minutes in a modified Carnoy's fluid (6 parts absolute alcohol, 3 parts chloroform, 1 part glacial acetic acid) and then transferred to absolute alcohol for dehydration. After 30 minutes in the alcohol, the tissues were cleared in cedarwood oil and imbedded in Fisher's tissuemat (m.p. 56–58° C.). Serial sections were cut in frontal and transverse planes at 8–10 microns and mounted on slides with Haupt's adhesive.

Pigment was dissolved from the sections prior to staining by placing the slides in a solution of acidified methyl alcohol (Ephrussi and Herold, 1944), which was substituted for absolute ethyl alcohol in the hydration which precedes staining. The most satisfactory stain used for the differentiation of the elements of the eye was Heidenhain's iron hematoxylin with ferric chloride as mordant and differentiating solution.

Whole mounts were prepared by fixing heads in ethylene glycol monoethyl ether for 15 minutes, clearing in xylol, and mounting in clarite. Eyes prepared in this way retained their pigment and were used for study of the external features of the mutants.

The analysis of the structural irregularities in all of the lozenge alleles was made by studying the serial sections of a number of different preparations for each mutant. Each slide was examined and the abnormalities were tabulated on the basis of the appearance of the structures in comparison to the wild-type compound eye. (See Table 1.) Every section on each slide was studied so that the most abnormal region could be selected as an indication of the extent of deviation from normal that occurred in that eye.

TABLE 1

A Comparison of the Abnormalities of the Ommatidia Produced by the Lozenge Alleles

(All values given are expressed in percentage based upon the total number of slides examined for each mutant. The most severe abnormality was taken in each case as an indication of the extent of the effect in each individual examined.)

		lz^{37}	lz	lz^{BS}	lz^{g}	lz^{34}	lz^{3}	lz^{y4}	lz^{sB}	lz^{s}	lz^{36}
	Total Number of Slides Examined	33	31	30	37	22	32	41	34	32	36
CORNEA	Fused area with some normal facets	100	100	100	100	100					
	No normal facets present						100	100	100	100	100
PILE	Present with each facet	82									
	Present but irregular	18	100	100	100	100	94	100			
	Absent						6		100	100	100
PSEUDOCONE	Present	88	67	73	32	45					
	Very abnormal or absent	12	33	27	68	55	100	100	100	100	100
PRIMARY PIGMENT CELLS	Present in normal position	88	67	73	32	45					
	Present in layer under the cornea	12	33	27	68	55	100	100	100	100	100
RHABDOMERE LENGTH	Normal length	52	7	20	8						
	Shorter than normal	48	93	80	92	100	100	100	100	100	100
RHABDOMERE SIZE AND NUMBER	Normal in diameter and number	70	10	17	8						
	Thicker than normal but normal number	15	48	13							
	Fused or very abnormal in shape	15	42	70	92	100	100	100	100	100	100
BASEMENT MEMBRANE	Present and normal	12	3								
	Present, abnormal cells in postretina	39	29	27	13						
	Present, disrupted by abnormal retinulae	49	68	73	87	91	12	5			
	Indistinct or absent					9	88	95	100	100	100
OMMATIDIAL ARRANGEMENT	Normal	52	7	7	5						
	Proper position but shorter than normal	45	67	93	95	73					
	Abnormal arrangement	3	16			27	100	100	100	100	100

RESULTS

The Wild-Type Compound Eye

The basic histological study of the normal compound eye of *Drosophila* was made by Johannsen in 1924. The features of the eye closely resembled those described by Hickson (1885), Lowne (1895), and Dietrich (1909) for the pseudocone type eyes of calyptrate flies. Krafka (1924) studied the normal eye structure and its development for comparison with the *Bar-eye* mutant. Other studies, which included descriptions of the normal compound eye, have been made by Richards and Furrow (1925), Cochrane (1937), Pilkington (1941), Waddington and Pilkington (1943), Steinberg (1943), and Miller (1950). Hertweck (1931) included a description of the compound eye in his detailed study of the nervous system and sense organs of *D. melanogaster*, as did Nolte (1950) in his work on the eye pigmentary system of *Drosophila* and Power (1943) in his description of the brain of *Drosophila melanogaster*.

The wild-type compound eye is composed of approximately 700 ommatidia of the pseudocone type (Figure 1), varying in length from the short centrally located ommatidia to the longer ones near the periphery (Figure 14). The corneal facets (c, Figure 1) are hexagonal in shape, with small hairs, or pile, present in rows between the facets in such a way that there are three hairs around each facet. Beneath the cornea are located the four glandular cells which form the pseudocone (ps). These cells secrete a semifluid material which fills the cuplike area of the pseudocone. During fixation the pseudocone cells shrink, so that generally in stained preparations these cells appear as four fine axial strands in the cup with their nuclei (pcn) oriented at the bases of the two primary pigment cells (ppc). The secondary pigment cells (spc) are present around the ommatidium, extending from the region of the pseudocone to the basement membrane. Their nuclei are located at approximately the same level as the pseudocone nuclei; a cross-section through this region (Figure 1, B) shows the manner in which the secondary pigment cells form a protective sheath of pigment around the visual cells. Such a section also reveals the manner in which the four nuclei of the pseudocone surround the tips of the seven rhabdomeres (rh).

The sensory rhabdome consists of a bundle of seven rhabdomeres which spread apart during fixation except at the ends. These rods extend along the median surface of the seven elongate retinula cells (Figure 1, C). Six of the retinulae have their nuclei (rn) at the upper portion of the cells, but the seventh retinula has become displaced and its nucleus (rn_7) is located nearer the basement membrane than the other six nuclei. A cross-section through the nuclei of the six regular retinulae (Figure 1, D) shows the small displaced seventh retinula with its smaller rhabdomere; a section somewhat lower (Figure 1, E), through the region of the seventh retinula nucleus, reveals that at this point the displaced retinula is as large as the other six cells.

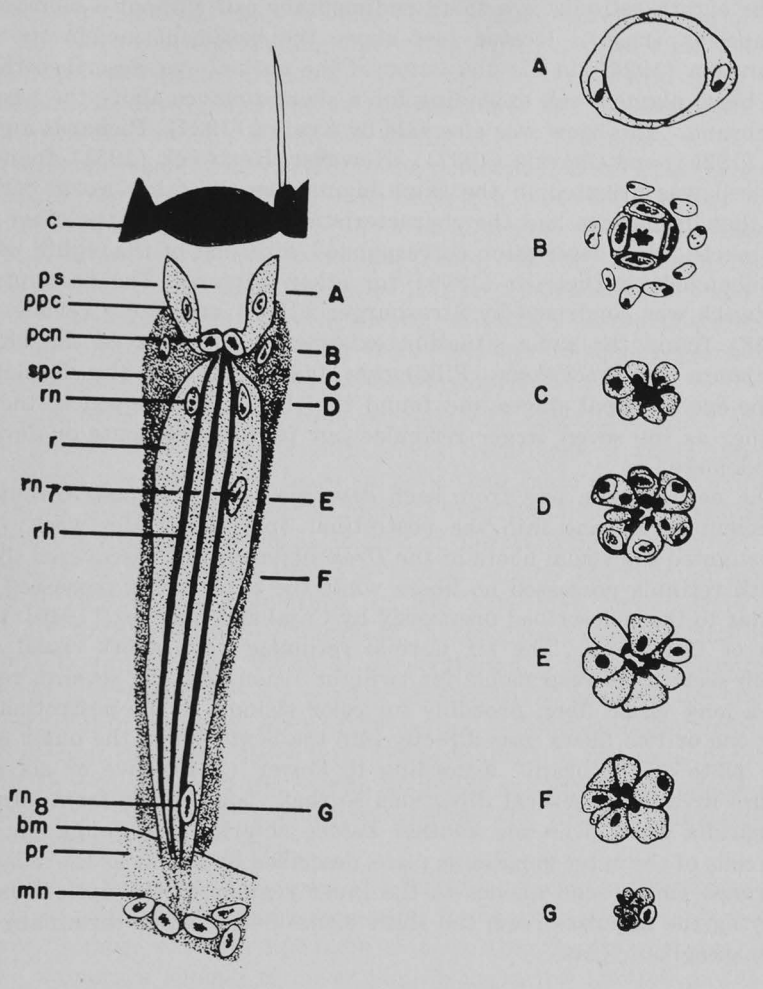


FIG. 1 WILD TYPE

FIG. 1. A single ommatidium from the wild type compound eye. Symbols: c, cornea; ps, pseudocone; ppc, primary pigment cell; pcn, pseudocone cell nucleus; spc, secondary pigment cell; rn, retinula nucleus; r, retinula; rn_7, seventh retinula nucleus; rh, rhabdomere; rn_8, eighth retinula nucleus; bm, basement membrane; pr, postretina; mn, monopolar neurones.

A—Cross-section through the pseudocone and primary pigment cells.

B—Cross-section through the pseudocone cell nuclei, showing the tips of the rhabdomeres and the nuclei of the secondary pigment cells.

C—Cross-section through upper portion of the retinulae, showing the relationship of the rhabdomeres to the retinulae.

D—Cross-section through the level of the nuclei of the six regular retinulae.

E—Cross-section through the level of the seventh retinula nucleus.

F—Cross-section through the seven retinulae, showing the small, displaced seventh retinula.

F—Cross-section through the nucleus of the eighth retinula.

The eighth retinula is a small rudimentary cell without a rhabdomere; its nucleus (rn_8) is located just above the basement membrane (bm). Johannsen (1924), in his discussion of the normal eye, described this cell as a basal pigment cell extending for a short distance above the basement membrane. This view was also held by Krafka (1924), Richards and Furrow (1925), and Chevais (1937). However, Hertweck (1931) found that this cell was oriented in the same manner as the other seven retinulae and that its nucleus had the characteristic appearance of the other retinular nuclei. His description corresponded with that of the eighth retinula cell depicted by Dietrich (1909) for other Diptera. The description of Hertweck was confirmed by Strasburger (1935) and Nolte (1950). Tate (1948) found the same situation existing in the eyes of the blow-fly, *Calliphora erythrocephala.* Pilkington (1941) studied the development of the eye in pupal stages and found that the cell developed in the same manner as the seven larger retinulae but failed to elongate or develop a rhabdomere.

The nerve fibers, one from each developed retinula, pass through the basement membrane into the postretinal (pr) area. Hertweck (1931) investigated the visual fibers of the *Drosophila* eye and discovered that the eighth retinula possessed no fibers while the other seven possessed fibers similar to those described previously by Cajal and Sanchez (1915) for the eyes of *Calliphora.* The six normal retinulae have short visual fibers, which seem to be responsible for twilight vision, and the seventh retinula has a long visual fiber, probably for color vision. In the postretinal area only one or two fibers pass directly into the next region, the outer ganglionic plate of the brain. According to Power (1943) five or six of the axones diverge in several directions so that visual fibers from adjoining ommatidia cross over one another before entering the ganglionic plate. The cells of the outer ganglionic plate, described by Power as the monopolar neurones (mn), send axones to the inner regions of the optic lobe, thus relaying the impulses from the short visual fibers which terminate in the outer ganglionic plate.

The Lozenge Mutants

The least severe of the lozenge alleles is lz^{37} for there is only a slight abnormality of the facets. Males have a small roughened area of fused or irregular facets, usually in the posterior region of the eye; whereas females of this genotype are almost normal in appearance. Sections through the length of the ommatidia of this mutant reveal that the abnormal condition of the eye is not restricted entirely to those areas in which the facets are abnormal, although in most cases these regions are the most severely affected. In some regions where the cornea is normal, the portion of the ommatidium proximal to the basement membrane has been disarranged by the presence of an abnormal retinular bundle located in the postretinal area. Such a section is shown in the photograph of lz^{37} (Figure 15) in which a single abnormal ommatidium is present. The rhab-

domeres are thickened and twisted, and in the postretinal region just below this abnormal ommatidium there is a darkly stained mass made up of abnormal ommatidial elements. The cornea, which pulled away from the ommatidia during fixation, is normal above the affected cells. Abnormal retinular bundles sometimes disrupted the rhabdomeres, preventing them from reaching downward to the postretinal area. Other abnormal cells below the basement membrane interfere with the passage of the nerve fibers from the retinulae to the area of the monopolar neurones. In the small areas where the facets are fused, several abnormal bundles may be grouped in the postretinal area (Figure 2) in such a way that their effect on the ommatidia is quite severe. Although severe abnormalities were found in the eyes of lz^{37}, they were restricted to small regions of the eye and the remainder of the ommatidia were normal.

The eyes of *lz* are rougher than normal, with patches of fused and abnormal facets. The pile is usually absent from some of these abnormal areas, which are larger and more variable than those of lz^{37}. Sections of the eyes of the *lozenge* genotype indicate that the abnormal condition of the cornea is the result of the same type of disarrangement as in lz^{37}. The photograph of *lz* (Figure 16) is a typical section showing an area of abnormal facets. The ommatidia in the abnormal region stain very darkly due to the presence of thickened rhabdomeres. An abnormal retinular bundle can be seen which is penetrating the basement membrane, and in the postretinal region a number of ommatidial elements are present. As illustrated in Figure 3, some of the retinular bundles in the postretinal region disrupt the nerve fibers from the ommatidia in the same manner as in lz^{37}. In regions where the cornea is smooth and flat the pseudocone cup is absent and there is a layer of pseudocone and primary pigment cells immediately below the cornea.

In *lozenge Bar-Stone* the fused and irregular facets are scattered over the surface of the eye in a manner similar to that of *lozenge*. This mutant, however, is more variable and more extreme than *lz;* its abnormal areas may be numerous enough to cover almost the entire surface of the eye, leaving only small groups of normal facets. In its more extreme form, lz^{BS} may produce a cornea which appears as a wettish sheet due to the fusion of a large number of facets. A typical section (Figure 17) reveals the degree of severity which may be present. The facets of the cornea exhibit abnormalities of shape and size which are indicative of the anomalies beneath. It can be seen from the photograph that regions in which there is irregular facet structure are also the areas in which retinular bundles are present in the postretinal area and penetrating the basement membrane. In those extreme regions where facets are absent the pseudocones are either very abnormal or absent. The primary pigment cells in such areas occur in a layer beneath the cornea and the basement membrane is indistinct. Retinulae are numerous in the postretinal area and the ommatidia above the postretina are abnormal in size and shape. Some

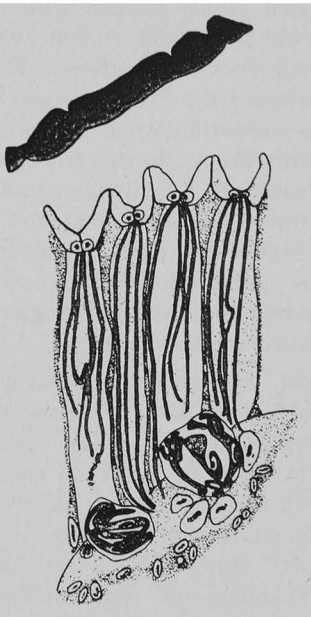


FIG. 2 lz^{37}

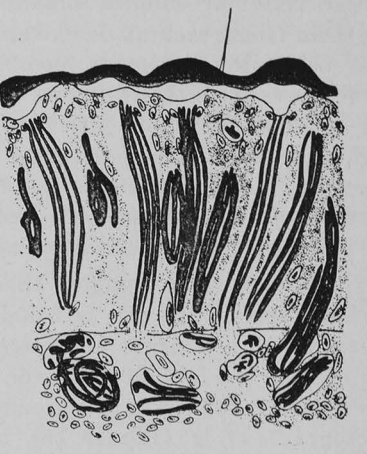


FIG. 3 lz

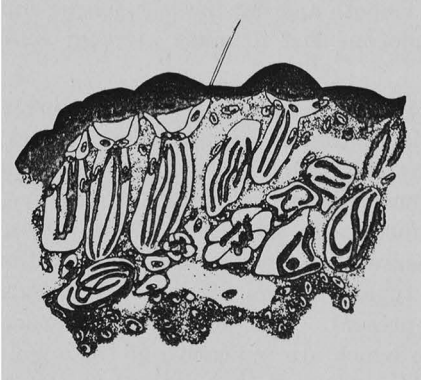


FIG. 4 lz^{BS}

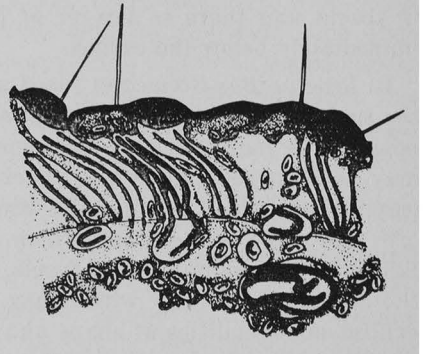


FIG. 5 lz^{g}

FIGS. 2–11. Sketches showing the elements of the ommatidia in the lozenge mutant, which are indicated in each individual figure.

FIGS. 2–5. Illustrate lozenge 37, lozenge, lozenge Bar-Stone, and lozenge glossy.

retinular bundles lie in a plane parallel to the surface of the eye so that their structures appear in cross-section (Figure 4).

The surface of the eye of lz^g has a characteristic glossiness due to large areas of fused facets. Normal facets are fewer and more scattered than in lz^{BS} and the hairs are present with the facets less frequently. The photograph (Figure 18) shows the typical abnormalities that characterize this mutant. A distinct variability in the size of the facets can be noted. Some facets, although normal in shape, are less than normal in size. Others are large, apparently covering an area which would be occupied normally by two facets. Small areas developed in which the cornea is flat on the external surface but appears to be thickened and overdeveloped in the region of the pseudocone. Longitudinal sections of lz^g (Figure 5) look very similar to those of lz^{BS} except that the affected areas are larger and severe irregularities of the pseudocone and primary pigment cells are more frequent.

The cornea of lz^{34} shows the most extreme deviation from the wild type among those alleles still retaining some normal facets. The surface of the eye is composed of large areas of fused and irregular facets, which give the eye a characteristic glazed appearance. The pile is present in small areas and normal facets are present only in small groups, usually several facets in two or three rows. The photograph of this mutant (Figure 19) illustrates the extent of the effect of the disruption of the ommatidia on the corneal facets. Some fused facets cover an area ordinarily occupied by two or more facets and small corneal patches are present which have failed to form true facets. Large regions appear in sections as thickened sheets in which the region normally occupied by the pseudocone cup is filled with the corneal secretion. The postretinal layer of ommatidial elements is more highly developed than in the previous mutants (Figure 6) and the basement membrane is ill-defined in most regions.

In lz^3 and lz^{v4} true facets fail to form and the surface of the eye has the general appearance of a wettish sheet, roughened in some areas by the formation of irregular facets. Hairs are present though sparse. The photographs of these two alleles (Figures 20 and 21) illustrate the extreme abnormal condition of the eyes. The irregularity in arrangement of the cells of the ommatidia and the obscurity of the basement membrane are apparent. In longitudinal sections lz^3 and lz^{v4} could not be distinguished since the abnormalities were of the same type and severity; however, these two alleles can be readily differentiated on the basis of the amount and distribution of pigment granules in the eyes. The basement membrane was either absent or very indistinct and the ommatidia were distorted and disoriented. Some of the retinulae lay in such a plane that oblique or cross-sections were obtained. The rhabdomeres became very thickened and twisted, and in many cases, stained very heavily. There was no indication of a pseudocone in slides of either mutant, the nuclei of the pseudocone and primary pigment cells appearing in a layer beneath the cornea.

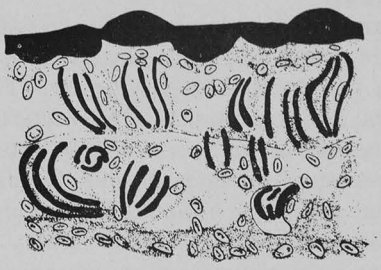


FIG. 6 Iz^{34}

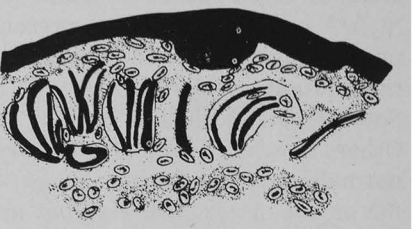


FIG. 7 Iz^{3}

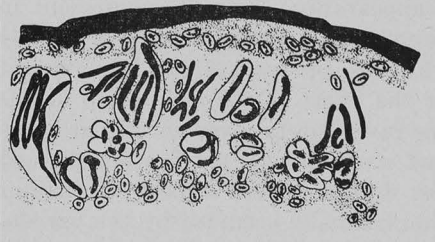


FIG. 8 Iz^{y4}

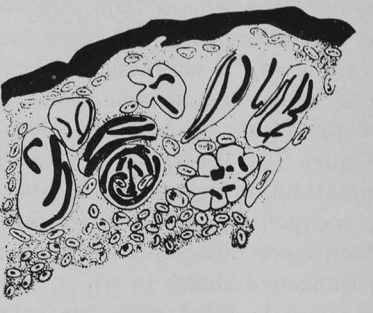


FIG. 9 Iz^{s}

FIG. 10 Iz^{SB}

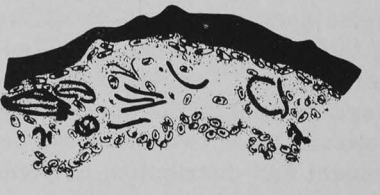


FIG. 11 Iz^{36}

FIGS. 6–11. Illustrate lozenge 34, lozenge 3, lozenge y-4, lozenge spectacle, lozenge spectacle-Bishop, lozenge 36.

The remaining three alleles, lz^{sB}, lz^{s}, and lz^{36}, are the most extreme of the lozenge series; in each mutant the surface of the eye is a smooth, hairless sheet, disrupted occasionally by small roughened areas. Of the three, lz^{36} tends to be the most severe, with roughness of the cornea greatly reduced. The photographs of these mutants (Figures 22, 23, and 24) show the gross disarrangement of all cells of the ommatidia. The most extreme manifestations of abnormality, which were seen in limited areas of the less severe mutants, are present throughout the eyes of these three alleles. The pigment cells and cone cells lie in a layer beneath the cornea. The basement membrane cannot be seen and the distinction of the postretinal area is difficult. Rhabdomeres can be seen in almost every plane; however, the retinulae are apparently still grouped in bundles in some regions as cross-sections reveal such an arrangement. Although it would be difficult to separate longitudinal sections of these alleles, lz^{36} is usually more extreme than either lz^{sB} or lz^{s} (see Figures 9, 10, and 11). The ommatidia are ordinarily not as severely disarranged in the latter two alleles as in lz^{36}; an examination of 36 slides of lz^{36} failed to indicate the presence of organized ommatidia. No distinction between lz^{s} and lz^{sB} could be made from studies of serial sections.

In the examination of the slides of the lozenge mutants, three individuals were found in which a different type of abnormality was observed. In two slides of lz^{3} and one of lz^{34}, the entire layer of ommatidia turned inward toward the brain along one margin of the eye. Both of these mutants have eyes which are narrower than normal so the abnormality may have developed as the result of insufficient space for expansion during growth of the cells. The cornea was absent in this ingrown region but pseudocone and primary pigment cells appeared in a layer above the retinulae.

The Lozenge Alleles as a Series

This study of the sections of the lozenge mutants has indicated that the severity of the structural abnormalities of the eyes corresponds in general to the external appearance of the cornea. Oliver (1947) placed the ten alleles in a series as follows: lz^{37}, lz, lz^{BS}, lz^{g}, lz^{34}, lz^{3}, lz^{v4}, lz^{sB}, lz^{s}, and lz^{36}, with lz^{37} showing only slight abnormalities of the facets and lz^{36} producing the most extreme condition, in which the cornea was a smooth hairless sheet. This seriation was based upon the appearance of the facets of the eye as revealed by direct observations of the external surface and by studies of the impressions of the eyes. The same gradation of the cornea was found in this study of sections. Typical sections of the cornea of the wild type eye and the lozenge alleles are shown in Figure 13. The facets of lz^{37} are normal except for small regions where fusion has occurred; the pile often appears to be normally arranged. In lz and lz^{BS} the fused areas are larger and the hairs of the facets are not arranged regularly. In lz^{g} and lz^{34} normal facets are very scarce and most of the cornea is a rough surface where true facets have failed to form. The cornea of lz^{3} and lz^{v4}

do not show normal facets and only a few scattered hairs are present. The surface of lz^{3} tends to be somewhat rougher than that of lz^{y4}. The remaining three alleles, lz^{s}, lz^{sB}, and lz^{36} have no pile and no normal facets. The corneal surfaces are rather smooth with occasional rough or thickened areas. The eyes of lz^{36} are usually smoother than the other two alleles, with fewer disruptions of the smooth corneal sheet.

In the tabulation of the slides of the various alleles (Table 1), the abnormalities of the cornea formed two distinct groups, one in which some normal facets appeared and the other in which no normal facets were present. Into the first category were placed the first five mutants of the series, lz^{37}, lz, lz^{BS}, lz^{g}, and lz^{34}. The remaining mutants, lz^{3}, lz^{y4}, lz^{s}, lz^{sB}, and lz^{36} are characterized by the complete absence of any true facets. The arrangement of the pile made it possible to separate lz^{37}, in which the distribution was normal in 82% of the slides, from the other four mutants having some normal facets. It was also possible to separate lz^{3} and lz^{y4} from lz^{s}, lz^{sB}, and lz^{36} by the presence of a few scattered hairs in lz^{3} and lz^{y4} and the complete absence in the other three mutants.

The structures of the ommatidia beneath the cornea showed different degrees of abnormality, thus making it possible to further separate the alleles on the basis of the severity of the genic effect on the visual cells.

In affected regions of the eyes, the pseudocone and primary pigment cells were usually present in their normal positions if there was an indication of facet formation above them. The pseudocone cups were often shallow and of an abnormal size with a corresponding deviation in the size of the facet. In those areas where no facet formation occurred the pseudocone cup was absent and the nuclei of the pseudocone and primary pigment cells were arranged in a layer beneath the cornea. In lz^{37} only 12% of the individuals showed this more extreme condition, thus separating this allele from lz, the next in the series, which showed the extreme condition in 33% of the slides. No significant difference was found between lz and lz^{BS}, which was composed of 27% with the more severe manifestation. The next two alleles fall into a distinctly different group in which the pseudocone and primary pigment cell nuclei are frequently in a layer beneath the cornea, with 68% in lz^{g} and 55% in lz^{34}. The remaining mutants showed the more severe condition in every individual examined, as would be expected since no true facets are formed in these alleles.

FIG. 12. Ommatidia of Wild Type and Mutants. A, Wild type eye; B—lz^{sB}, Ten retinulae and enlarged rhabdomeres present; C—lz^{g}, Normal number of retinulae present but rhabdomeres enlarged; D—lz^{3}, Nine retinulae and enlarged rhabdomeres present; E—lz^{g}, Nine retinulae and slightly enlarged rhabdomeres; F—lz^{34}, Normal number of retinulae but cells are irregularly arranged and rhabdomeres are enlarged; G—lz^{sB}, Ten retinulae present and portions of at least 12 rhabdomeres; H—lz^{s}, Eight abnormally shaped retinulae and seven rhabdomeres; I—lz^{37}, Eight retinulae and slight abnormality in shape of some rhabdomeres; J—lz^{BS}, Eight retinulae and nine rhabdomeres; K—lz^{y4}, Retinulae fused and rhabdomeres twisted so that some were cut in oblique plane; L—lz^{BS}, Seven retinulae and rhabdomeres, irregular arrangement of the retinulae and abnormal size of rhabdomeres; M—lz^{y4}, Seven retinulae with twisted rhabdomeres; N—lz^{3}, Reduced number of retinulae and abnormally large rhabdomeres; O—lz^{36}, Group of four retinulae with abnormal rhabdomeres; P—lz^{y4}, fused with portions of six rhabdomeres.

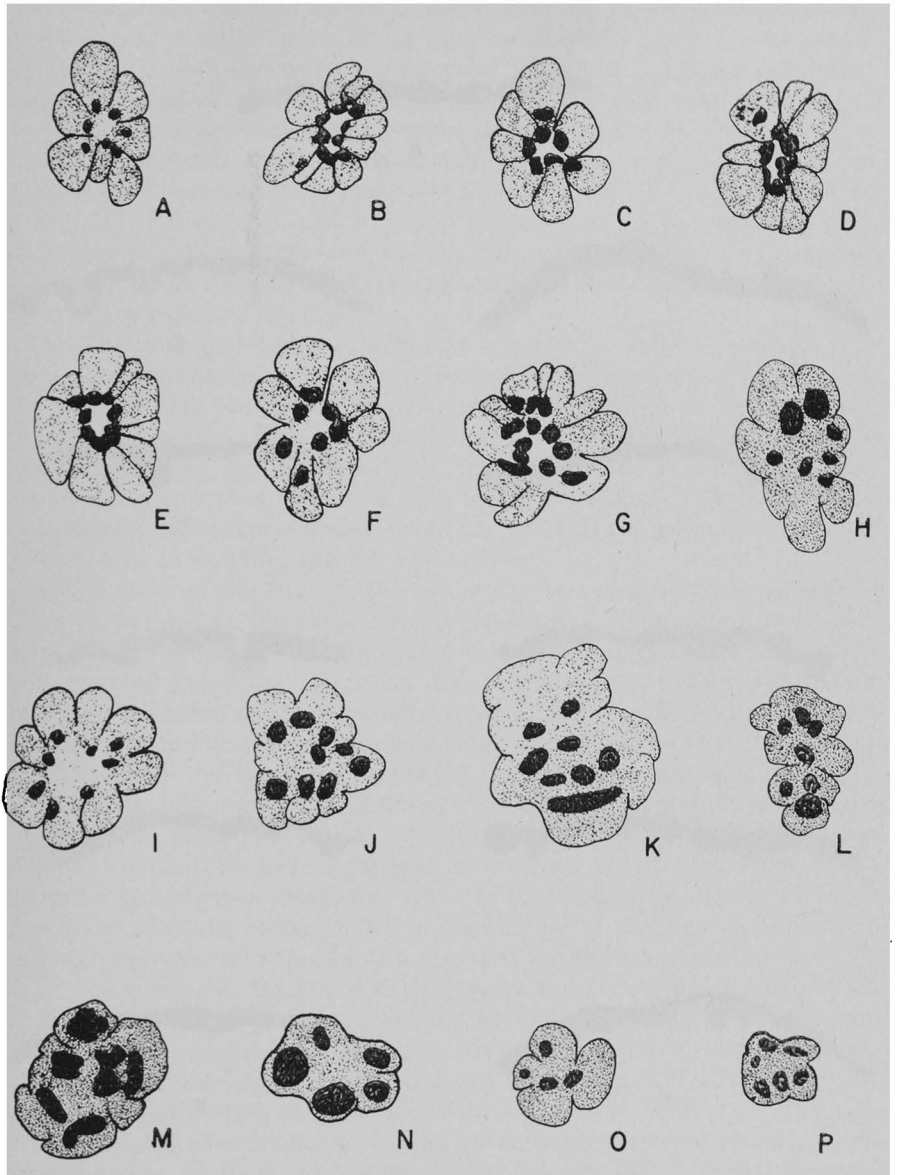


FIG. 12 OMMATIDIA OF WILD TYPE AND MUTANTS

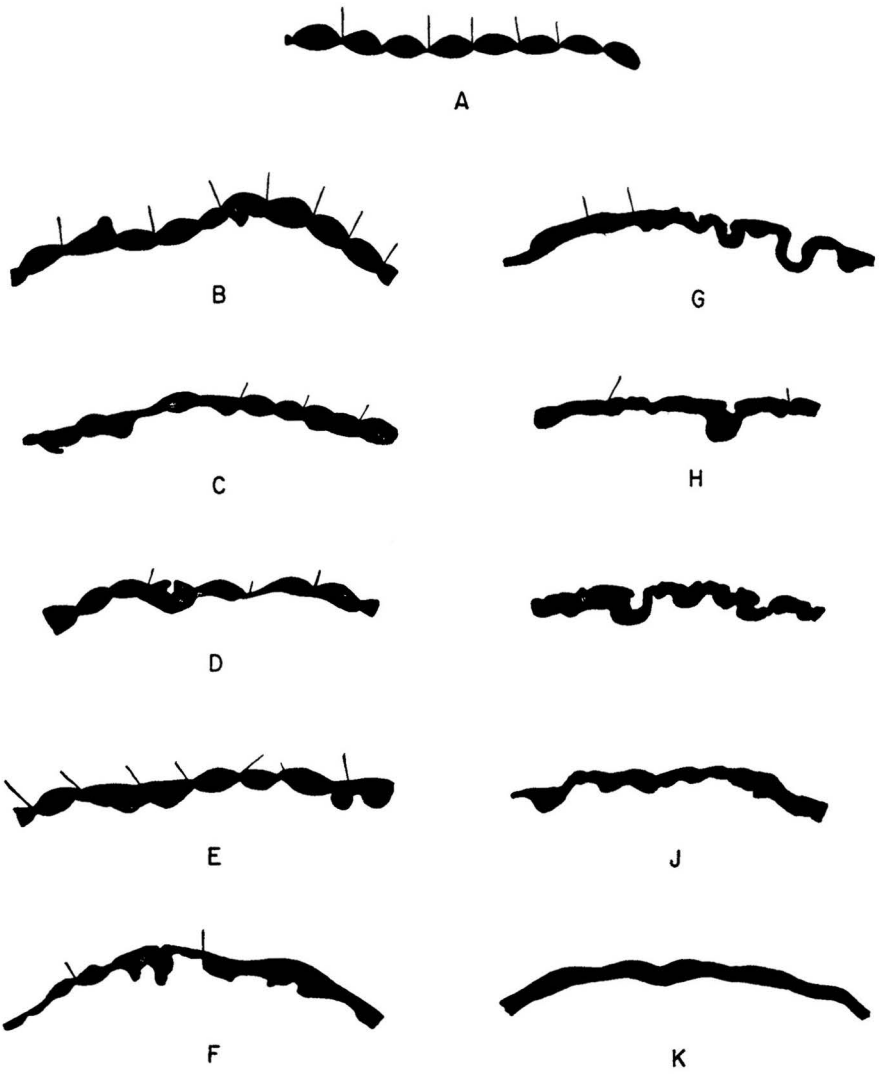


FIG. 13 CORNEA OF WILD TYPE AND MUTANTS

FIG. 13. Cornea of Wild Type and Mutants. A, Wild type; B, lz^{37}; C, *lz;* D, lz^{BS}; E, lz^{E}; F, lz^{34}; G, lz^{3}; H, lz^{v4}; I, lz^{s}; J, lz^{sB}; K, lz^{36}.

The abnormalities that occurred in the retinulae of the ommatidia were detected most readily by studying the rhabdomeres. In abnormal areas the rhabdomeres were sometimes normal in length but more frequently were shorter than normal. In lz^{37} the abnormal area was made up of rhabdomeres of approximately normal length in 52% of the cases. The remaining mutants showed short rhabdomeres in a large majority of the slides with this more severe condition present in 100% of the individuals of lz^{34}, lz^{3}, lz^{y4}, lz^{sB}, lz^{s}, and lz^{36}. An examination of the camera lucida drawings (Figures 2–11) and the photographs (Figures 15–24) shows this variation in the length of the rhabdomeres, with the resultant differences in the depth of the eye.

The rhabdomeres varied in size and number as well as in length. In those abnormal areas in which the ommatidial structure was very nearly like that of the wild type, the rhabdomeres appeared to be normal in number and in size. This was determined by examining the ommatidia in both longitudinal and cross-sectional regions of the slides. In lz^{37} the rhabdomeres appeared normal in 70% of the individuals. The other three alleles which had normal rhabdomeres, *lz*, lz^{BS}, and lz^{g}, showed much lower percentages, 10%, 17%, and 8% respectively.

In the more severe regions the rhabdomeres appeared to be thickened although present in the usual number of seven. The study of serial sections of these abnormal cells indicated that the thickened structures probably resulted from the failure of the retinulae to lengthen sufficiently during development. The most severe condition was found in some specimens of all the mutants; the rhabdomeres were fused so that the number was abnormal and the shape indicated that twisting and bending of the rhabdomeres had occurred. Cross-sections through ommatidia which normally would show the rosette of seven retinulae indicated that some abnormal ommatidia were composed of as many as ten cells, while others showed a reduction in number to three or four cells; the number of retinulae present usually corresponded to the number of rhabdomeres observed, although in some cases there was a disparity between the two (Figure 12). In lz^{37}, 15% had the thickened rhabdomeres and 15% showed some fusion of the rhabdomeres. In *lz*, where only 10% were normal, the thick rhabdomeres were present in 48% of the individuals, and 42% showed fusion. The fusion of the rhabdomeres occurred in 70% of lz^{BS} with only 13% showing the thickened rhabdomeres with the normal number of retinulae. In lz^{g}, 92% of the individuals showed the twisted and fused condition, and this was true in 100% of the remaining alleles. Thus, significant percentage differences in this category make possible the separation of lz^{37}, *lz*, lz^{BS}, and lz^{g} from one another with a high frequency of success.

The abnormalities in the region of the basement membrane were found to be of several types. The most nearly normal condition was one in which there was some disarrangement of the pseudocones or retinulae above the basement membrane but the region of the membrane and the postretinal area was normal. This condition was present in two mutants only, in lz^{37}

12% of the time, and in *lz* 3% of the time. In all of the other alleles there was some disarrangement in the region of the basement membrane and postretina. In 27% of the lz^{BS} and 13% of the lz^{g} the membrane was normal in appearance but there were some abnormal retinulae in the postretinal area. This condition was present in 29% of the *lz* individuals and 39% of the lz^{37}. A more severe modification, in which the basement membrane was disrupted in certain places by retinulae penetrating the membrane, occurred in individuals of all the alleles except lz^{sB}, lz^{s}, and lz^{36}. In these three mutants there was no distinct membrane present in any of the individuals examined. In lz^{37}, *lz*, lz^{BS}, and lz^{g} a basement membrane could be distinguished in every individual and the greatest degree of abnormality was this disruption of the membrane, occurring more frequently in the mutants with the greater abnormality of the corneal surface. In lz^{34}, 91% of the individuals had the basement membrane disrupted by retinulae; this factor alone is not significantly different from the percentage for lz^{g} until one considers that this is the least severe condition in lz^{34}, with the remaining 9% of the individuals possessing no distinct membrane at all, whereas in lz^{g}, the disruption of the basement membrane in 87% of the cases was the most severe modification found. In lz^{3} and lz^{y4} the absence of a distinct membrane is characteristic of 88% of the former mutant and 95% in the latter.

In the abnormal regions of the eyes of each of the mutants the arrangement of the ommatidia in relation to the corneal surface and the brain varied with the severity of cellular modifications. In lz^{37}, where the lozenge effect is the most moderate, the ommatidia were in their normal position and of normal length in 52% of the slides, were in their normal position, but shorter than normal in 45% of the individuals, and showed an abnormal arrangement only 3% of the time. In *lz*, where the effect is somewhat more severe, the normal condition was found in only 7% of the individuals and 67% showed the intermediate condition. The abnormal areas of lz^{BS} had normally arranged ommatidia 7% of the time and the remaining 93% were shorter than normal though in the proper location. No significant difference was found in this characteristic for lz^{g}. In 73% of the lz^{34} the ommatidia were in the proper position but were shorter than normal, and the remaining 27% were disarranged to such an extent that sections which cut through the length of the normal ommatidia cut the cells of the abnormal areas in many different planes. The remaining alleles, in which no normal facets formed, had this abnormal condition in 100% of the individuals.

Significant differences in percentages for the various types of abnormalities make it possible to arrange the alleles in a rather definite series from lz^{37}, which is almost normal in phenotype, to lz^{36}, which is the most severe of the group. The basis of separation of alleles which are phenotypically similar is shown in Table 2, which is derived from the percentages of Table 1. The separation of lz^{37} from *lz* can be made on the basis of all of the percentages obtained except those for fused areas of the cornea.

Distinction between the cornea of these two mutants can be readily made, however, by examination of the external surface of the eye. The differences in percentages between *lz* and lz^{BS} were not significant except in the size and number of rhabdomeres. Fused and abnormal rhabdomeres were much more frequent in lz^{BS} than in *lz*. The basement membrane was normal in 3% of the *lz* whereas no individuals of lz^{BS} were normal in this respect; however, the percentages in other abnormalities of the basement membrane were too similar to distinguish between the alleles on this basis.

TABLE 2

The Segregation of the Lozenge Alleles on the Basis of Ommatidial Abnormalities (The grouping in this table is based on the significant similarities or differences in percentages obtained in Table 1.)

Cornea	lz^{37}	lz	lz^{BS}	lz^{g}	lz^{34}	lz^{3}	lz^{y4}	lz^{sB}	lz^{s}	lz^{36}
Pile	lz^{37}	lz	lz^{BS}	lz^{g}	lz^{34}	lz^{3}	lz^{y4}	lz^{sB}	lz^{s}	lz^{36}
Pseudocone	lz^{37}	lz	lz^{BS}	lz^{g}	lz^{34}	lz^{3}	lz^{y4}	lz^{sB}	lz^{s}	lz^{36}
Primary Pigment Cells	lz^{37}	lz	lz^{BS}	lz^{g}	lz^{34}	lz^{3}	lz^{y4}	lz^{sB}	lz^{s}	lz^{36}
Rhabdomere Length	lz^{37}	lz	lz^{BS}	lz^{g}	lz^{34}	lz^{3}	lz^{y4}	lz^{sB}	lz^{s}	lz^{36}
Rhabdomere Size and Number	lz^{37}	lz	lz^{BS}	lz^{g}	lz^{34}	lz^{3}	lz^{y4}	lz^{sB}	lz^{s}	lz^{36}
Basement Membrane	lz^{37}	lz	lz^{BS}	lz^{g}	lz^{34}	lz^{3}	lz^{y4}	lz^{sB}	lz^{s}	lz^{36}
Ommatidia Arrangement	lz^{37}	lz	lz^{BS}	lz^{g}	lz^{34}	lz^{3}	lz^{y4}	lz^{sB}	lz^{s}	lz^{36}

Similarly, it is difficult to distinguish the more extreme forms of lz^{BS} and some individuals of lz^{g} from the study of sections. A tabulation of the conditions on a number of slides, however, shows a significant difference in several characteristics. The percentages obtained on the pseudocone and primary pigment cell abnormalities are almost reversed, with lz^{BS} showing the least severe condition 73% of the time and lz^{g} having the abnormal condition in 68% of the slides. Distinction can be made also on the basis of percentages obtained from the size and number of rhabdomeres.

The distinction between lz^{g} and lz^{34} is based primarily on abnormalities of the basement membrane region and the arrangement of the retinulae. Small differences in percentages occur in the anomalies of the rhabdomeres, but they would not be considered significant unless a much larger number of slides had been examined. The next two alleles, lz^{3} and lz^{y4}, are easily separated from lz^{34} on the percentages obtained. Significant differences occur in corneal abnormalities, disarrangement of the pseudocone and primary pigment cells, disruption of the basement membrane, and the arrangement of the retinulae.

From a study of sections only, no separation could be made between lz^{3} and lz^{y4}; however, these two can be separated easily on the basis of the amount and distribution of the red and brown eye pigments. The remaining three alleles form a group which is distinct from lz^{3} and lz^{y4} on

the basis of the pile. Hairs are absent from the cornea of lz^{s}, lz^{sB}, and lz^{36} but they are present, though sparse, on the surface of the eyes of lz^{3} and lz^{y4}. A small difference in percentages was obtained in the study of the basement membrane; the membrane was seen in a small number of individuals of lz^{3} and lz^{y4} but could never be located distinctly in the remaining alleles. No distinction could be made between lz^{s}, lz^{sB}, and lz^{86} from sections although the cornea of lz^{36} usually shows less roughness than either of the other alleles.

DISCUSSION

A comparison of the seriations of the alleles based on a histological study clearly indicates that the phenotypic abnormalities of the cornea bear a direct relationship to the structural abnormalities of the ommatidia. This was indicated for lz^{s} by Waddington and Pilkington (1943) and is shown in this study for ten different lozenge alleles. The seriation of the alleles by Oliver (1947) on the basis of facet irregularities shows a high degree of correlation with the series derived from this study. Those mutants most closely related phenotypically also resemble one another in the abnormalities of the ommatidia. The production of a normal facet seems to be dependent upon the development of a normal ommatidium beneath the cornea, and the degree of abnormalities produced during differentiation determines the extent of the facet irregularities.

The arrangement of the alleles by their pigment distribution was included in Oliver's study also. He found that a relationship existed between facet irregularity and pigmentation but the linear arrangement of the alleles based upon these two factors showed some striking differences. The alleles, lz^{37}, lz, lz^{BS}, lz^{g}, and lz^{34}, arranged in this order on increasing facet irregularities, also show an increasing darkness in the eye color. The next two alleles lz^{3} and lz^{y4}, do not fit into the series in the same way for pigmentation as for structural abnormalities; lz^{y4} is darker than lz^{34} but lz^{3} has a reduced amount of pigment. The remaining three alleles would belong in a group with lz^{3}.

It seems quite possible that one important factor in the irregularity of pigment distribution is the disarrangement of the ommatidia. The primary pigment cells show a considerable degree of modification and disarrangement in the lozenge alleles, and the secondary pigment cells are altered in their positions whenever the retinulae are abnormally placed. This would considerably alter the distribution and location of the red and brown pigment granules. The results obtained by Green (1948) in his analysis of the red pigment in seven lozenge alleles indicate that the quantity of pigment formed is not the only factor involved, and he suggests that differences in distribution of the pigment are also essential in the phenotypic dissimilarities between mutants which form almost the same amount of pigment. Histological studies on the lozenge series to determine the irregularities in the distribution of the red and brown pigment granules will aid in solving this problem.

The study made by Anderson (1945) on the infertility of the lozenge females did not produce a seriation for the different alleles. All of the alleles except lz^{37} lack the spermathecae and parovaria, and the only seriation was within the lz^{37} mutant where the degree of effect on the genitalia was related to the extent of fertility. A histological study of the develment of lz^{g} indicated that the abnormal genitalia resulted from the lack of proper cell differentiation in the genital disc during development.

It is also known that lz^{s} and lz^{sB}, which are apparently identical in their effects on eye color and ommatidial abnormalities produce different results in compounds formed with other lozenge alleles (Oliver, 1945; Cummings, 1946).

The various studies which have been carried out on different aspects of the problem of the lozenge complex indicate that the different phenotypic expressions of an allele are interrelated for some characteristics but independent for others. There is a definite relationship between facet irregularities and ommatidial abnormalities and probably the same exists for pigment distribution. Thus, these factors are dependent upon the extent of the deviation from normal development during differentiation of the ommatidial cells. The infertility of the females is also dependent on the cellular differentiation in the imaginal genital disc. However, since no seriation exists for anomalies of the female genital ducts, and all alleles except lz^{37} show the same degree of morphological effect there must be an independent action in the production of the degree of facet abnormality and the infertility of the homozygous females. Although infertility and facet irregularities can definitely be correlated with cellular abnormalities, other factors must be considered in an explanation of the irregularities in pigment distribution, quantitative differences in the amount of red and brown pigments, and the variations in the interactions between various alleles of the lozenge complex.

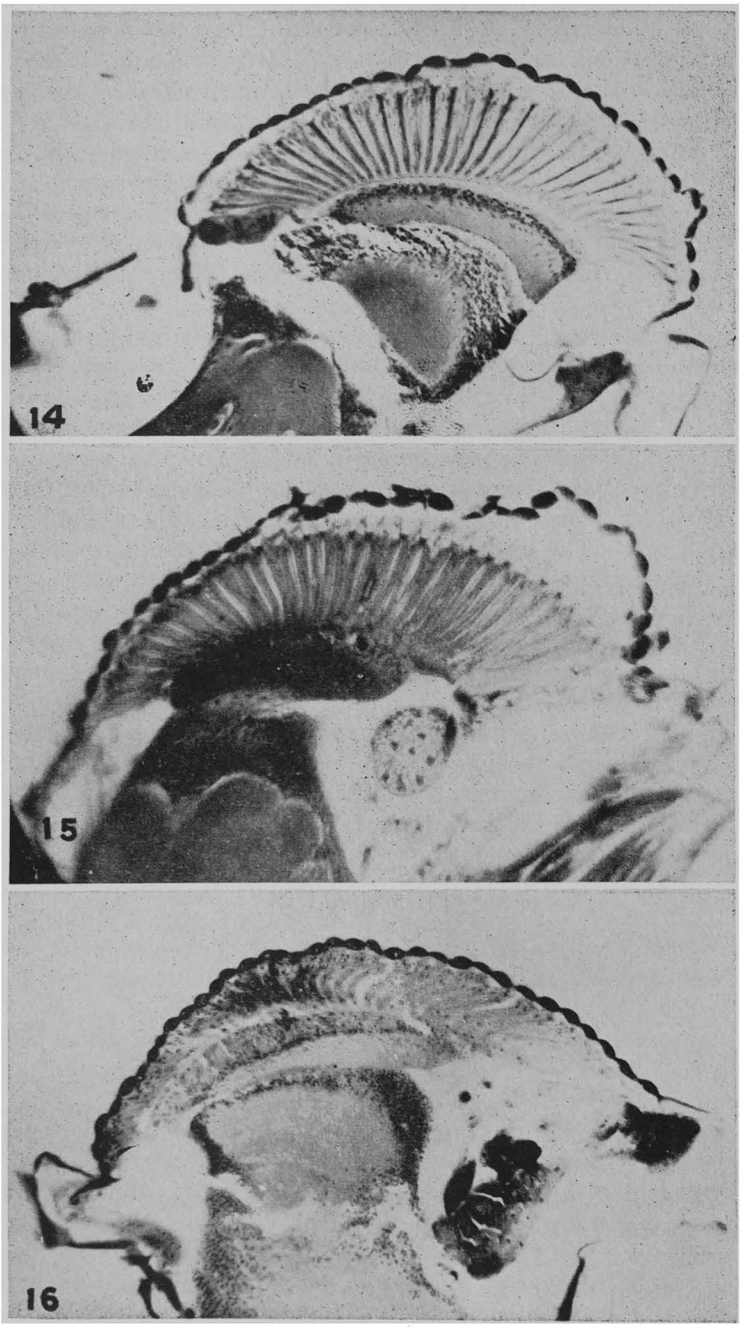
SUMMARY

Histological sections of wild type *Drosophila melanogaster* and ten lozenge alleles were studied to determine the type and extent of ommatidial irregularities produced by the different mutants.

The abnormalities of the alleles were tabulated on the basis of the effect on the corneal surface, the pile, the pseudocone and primary pigment cells, the retinulae and rhabdomeres, and the basement membrane.

The percentages obtained from the tabulation allowed a linear arrangement of the alleles, from lz^{37} with only slight abnormalities to lz^{36} with severe irregularities. This arrangement agreed in general with the seriation proposed by Oliver for the same alleles on the basis of facet irregularities.

It was indicated that the abnormalities of the ommatidia may also be one factor in the variations in pigment distribution among the mutants. It was further indicated that genic control of cellular differentiation during development is sufficient to account for the facet irregularities and the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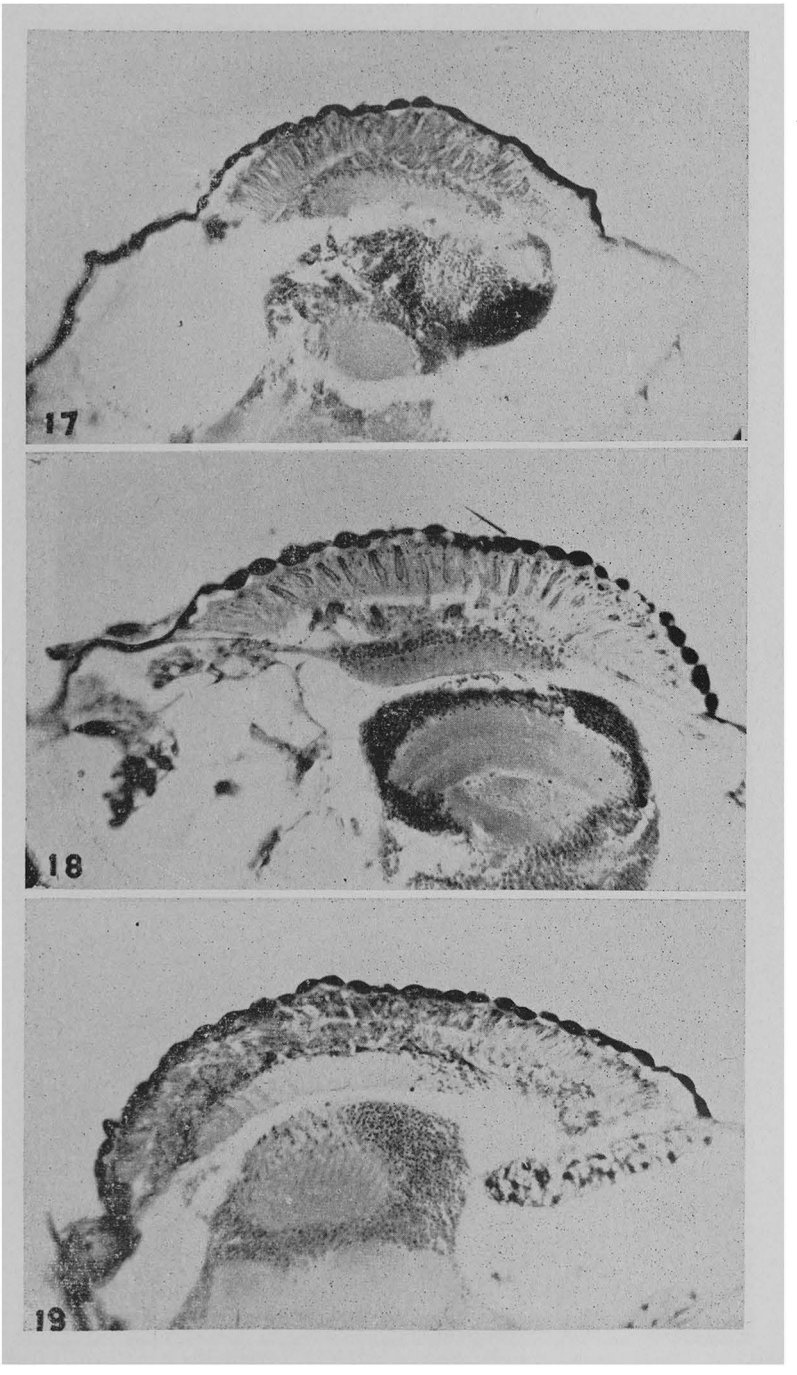
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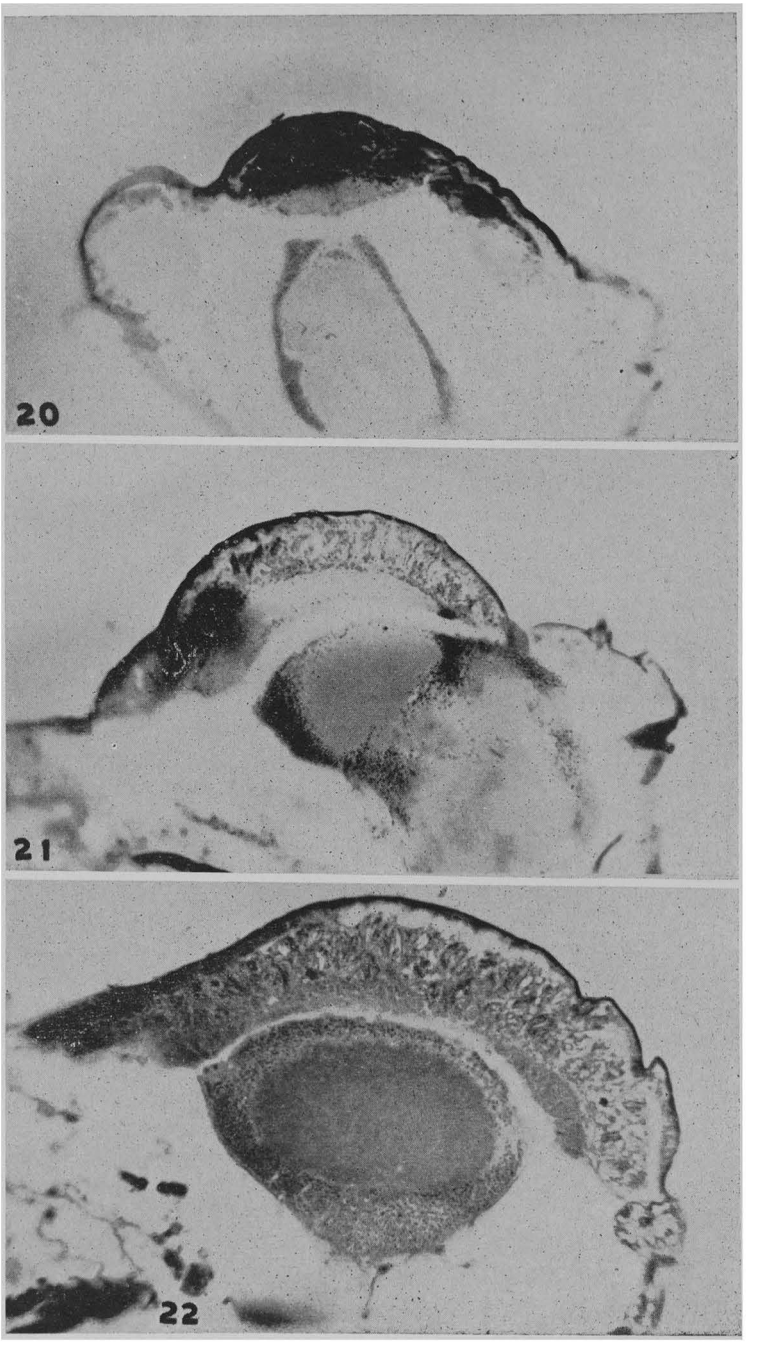
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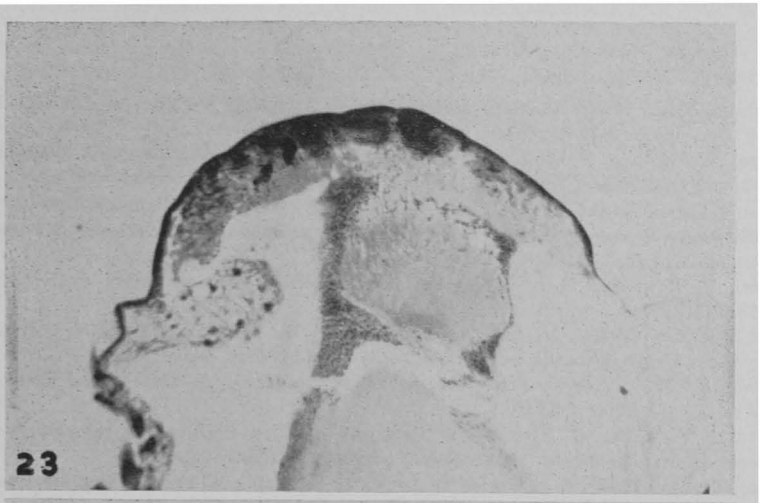

FIGS. 14–24. Photographs of section of the compound eye in the wild type and lozenge mutants. Fig. 14, wild type; Fig. 15, lozenge 37; Fig. 16, lozenge.

FIG. 17. Lozenge Bar-Stone; Fig. 18, Lozenge glossy; Fig. 19, Lozenge 34.

FIG. 20. Lozenge 3; Fig. 21, Lozenge y4; Fig. 22, Lozenge spectacle. FIG. 23. Lozenge spectacle-Bishop; Fig. 24, Lozenge 36.

infertility of the homozygous females but other factors must be considered to satisfactorily explain the quantitative differences in pigment deposition in the eyes and the differences in the interactions between the alleles.

BIBLIOGRAPHY

Anderson, Ray. 1945. A Study of the Factors Affecting Fertility of Lozenge Females of *Drosophila melanogaster*. Genetics, 30:280–297.

Bridges, C. B. and Brehme, K. S. 1944. The Mutants of *Drosophila melanogaster*. Carne. Inst. Wash. Publ., 552:118–119.

Ramon y Cajal, S. and D. Sanchez. 1915. Contribucion al Conocimiento de los Centros Nerviosos de los Insectos. Trabajos del Laboratorio de Investigaciones biologicas. Univ. Madrid, 13:1–167.

Casteel, D. B. 1929. Histology of the Eyes of X-rayed Drosophila. Jour. Exp. Zool., 53:373–385.

Chen, Tse-Yin. 1929. On the Development of the Imaginal Buds in Normal and mutant *Drosophila melanogaster*. Jour. Morph., 47:135–201.

Chevais, S. 1937. Sur la Structure des Yeux Implantes de *Drosophila melanogaster*. Arch. Anat. Micro., 33:107–112.

Cochrane, F. 1937. A Histological Analysis of Eye Pigment Development in *Drosophila pseudoobscura*. Proc. Roy. Soc. Edinb., 57:385–399.

Cummings, K. 1946. A Study of a Series of Multiple Alleles at the Lozenge Locus in *Drosophila melanogaster*. Thesis for M.A. Univ. of Minn., June, 1946.

Dietrich, W. 1909. Die Facettenaugen der Dipteren. Zeitschr. f. wiss. Zool., 92:465–539.

Ephrussi, B. and Herold, J. L. 1944. Studies of Eye Pigments of *Drosophila*. I. Methods of Extraction and Quantitative Estimation of the Pigment Components. Genetics, 29:148–175.

Gottschewski, G. 1936. Quantitative und qualitative Unterscheide innerhalb einer Allelenreihe bei *Drosophila melanogaster*. Zool. Anz. Suppl. 9:104–112.

Green, M. M. 1948. Red Eye Pigment Determination in the Lozenge Allelic Series of *Drosophila melanogaster*. Amer. Nat. 82:188–192.

Hertweck, Heinrich. 1931. Anatomie und Variabilitat des Nervensystems und der Sinnesorgane von *Drosophila melanogaster*. Zeitschr. wiss. Zool., 139:559–663.

Hickson, S. J. 1885. The Eye and Optic Tract of Insects. Quarterly Jour. of Microscopical Sci., Series 2, Volume 25.

Johannsen, O. A. 1924. Eye Structure in Normal and Eye-mutant Drosophilas. Jour. Morph. and Physiol., 39:337–349.

Krafka, J. 1924. Development of the Compound Eye of *Drosophila melanogaster* and its Bar-eyed Mutants. Biol. Bull., 47:143–148.

Lowne, B. T. 1895. The Anatomy, Physiology, Morphology and Development of the Blow-fly. Volume 2:501–582.

Miller, Albert. 1950. The Internal Anatomy and Histology of the Imago of *Drosophila malanogaster*. Biology of Drosophila. John Wiley & Sons: New York. Pages 420–591.

Nolte, D. J. 1950. The Eye Pigmentary System of *Drosophila:* the Pigment Cells. Jour. Genetics, 50:79–99.

Oliver, C. P. 1945. Four lozenge alleles phenotypically alike which react differently with their other alleles. Genetics, 30:16.

Oliver, C. P. 1947. Interrelationship between Eye Color and Facet Arrangement in Lozenge Alleles of *Drosophila melanogaster*. Univ. of Tex. Publ., 4720:167–184.

Oliver, C. P. and Green, M. M. 1944. Heterosis in Compounds of Lozenge Alleles in *Drosophila melanogaster*. Genetics, 29:331–347.

Pilkington, R.W. 1941. Facet Mutants of *Drosophila*. Proc. Zool. Soc. London, Series A. 111:199–222.

Power, M. E. 1943. The Brain of *Drosophila melanogaster*. Jour. Morphol., 72:517–559.

Richards, M. H. and Furrows, E. Y. 1925. The Eye and Optic Tract in Normal and "eyeless" *Drosophila*. Biolog. Bull., 48:243–258.

Steinberg, Arthur G. 1943. The Development of Wild Type and Bar Eyes of *Drosophila melanogaster*. Canadian Jour. of Research. Section D. 21:277–283.

Strasburger, E. H. 1935. *Drosophila melanogaster* Meig. Eine Einfuhrung in den Bau und die Entwicklung. Berlin: Julius Springer. 60 pages.

Tate, P. 1948. The Structure of Normal and Mutant Eyes in the Blow-fly *(Calliphora erythrocephala)* and the Development of Eye Pigment during Development. Jour. Genetics., 48:338–342.

Waddington, C. H. and Pilkington, R. W. 1943. The Structure and Development of Four Mutant Eyes in *Drosophila*. Jour. Genetics., 45:44–50.